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Abstract—Blacktail comber (*Serranus atricauda* Günther) is a commercially important species in the Canary Islands fisheries. A total of 425 individuals were collected and histological techniques were used to investigate the reproductive data. Results indicated that the spawning season occurred throughout the year, peaking between March and July. Individuals reached 50% maturity at 19.3 cm TL and 95% at 33.1 cm TL. Batch fecundity estimates ranged from 21,774 to 369,578 oocytes per spawning event for specimens from 22.2 to 39.8 cm TL. Blacktail comber was estimated to spawn 42 times/year and 26.5% of individuals spawned, on average, every 3.8 days. Estimates of potential annual fecundity for the species ranged from 0.91 to 15.5 million oocytes, and an average of 5.1 ±4.1 million.

Spawning season, maturity sizes, and fecundity in blacktail comber (*Serranus atricauda*) (Serranidae) from the eastern-central Atlantic

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Fish of the genus *Serranus* are synchronous hermaphrodites; male and female tissues are simultaneously functional (Smith, 1965). The most common style of reproduction is serial monogamy, in which individuals are solitary during the day before pairing up and spawning in the late afternoon (Fischer, 1986). During spawning one fish in each pair functions as a male and the other as a female and cross-fertilization occurs. This special characteristic, and the possibility of self-fertilization, have encouraged detailed studies of the gonad structure of the genus *Serranus* (Atz, 1965; Fishelson, 1970; Reinboth, 1970; Febvre et al., 1975; Zanuy, 1977; Bruslé, 1983; Abd-el-Aziz and Ramadan, 1990; García-Díaz et al., 1997, 2002), although knowledge of other reproductive features is scarce.

Models of dynamic population used in the management of fishery resources and in biological studies of fish require knowledge of the reproductive system (Koslow et al., 1995). This includes gonad morphology (external and cellular description of the ovary and testis), reproductive pattern (hermaphoditism or gonocorism), reproductive behavior, reproductive cycle, spawning season duration, size at maturity, sex ratio, size at sexual transition, and fecundity. Of all these reproductive features, fecundity is the most difficult biological parameter to obtain, although it is of interest to fishery scientists both as a critical parameter for stock assessments based on egg production methods and as a basic aspect of fish biology and population dynamics. To calculate fecundity, fish are divided into determinate and indeterminate spawners (Hunter and Macewicz, 1985a; Hunter et al., 1992). With indeterminate spawners, multiple or serial batches are noted, and spawning may take place many times during a protracted spawning

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season. Therefore fecundity is very difficult to determine before the onset of the spawning season because 1) there is no way to differentiate between the oocytes that are going to be shed during the next season from those which will remain for future seasons, and 2) the number of reserve oocytes that will mature during the next spawning season cannot be predetermined (Hunter and Goldberg, 1980; Hunter et al., 1985). To estimate indeterminate annual fecundity, the mean number of eggs per batch and spawning frequency throughout the spawning season must be calculated. Owing to the complexity of obtaining these data, fecundity studies are normally limited to determinate spawners or pelagic species of substantial economical interest (Hunter and Goldberg, 1980; Hunter and Macewicz, 1985a; Karlou-Riga and Economidis, 1997; Murua et al., 1998).

The blacktail comber (*Serranus atricauda* Günther, 1874), is a littoral (3–150 m) benthic species, ranging throughout the eastern central Atlantic (from the Bay of Biscay to Mauritania, the Azores, Madeira. and the Canary Islands) and in the western Mediterranean. It is, therefore, a species of wide distribution and commercial interest in many regions (Bauchot, 1987; Smith, 1990). In the Canary Islands, it is an economically important species for small-scale inshore fisheries (Pérez-Barroso et al., 1993). García-Díaz et al. (1999, 2002) determined that this species is a functional simultaneous hermaphrodite. The ovary is classified as asynchronous, i.e., various stages of oocyte development occur simultaneously (primary growth stage, yolk vesicle formation, vitellogenesis, oocyte maturation, and mature egg). The histological structure of the gonad and of the sperm indicates that this species is an externally fertilizing teleost.

The objective of this article is to increase our understanding of the reproductive biology of the blacktail comber in the Canary Islands by estimating its spawning season duration, size at maturity, and fecundity.

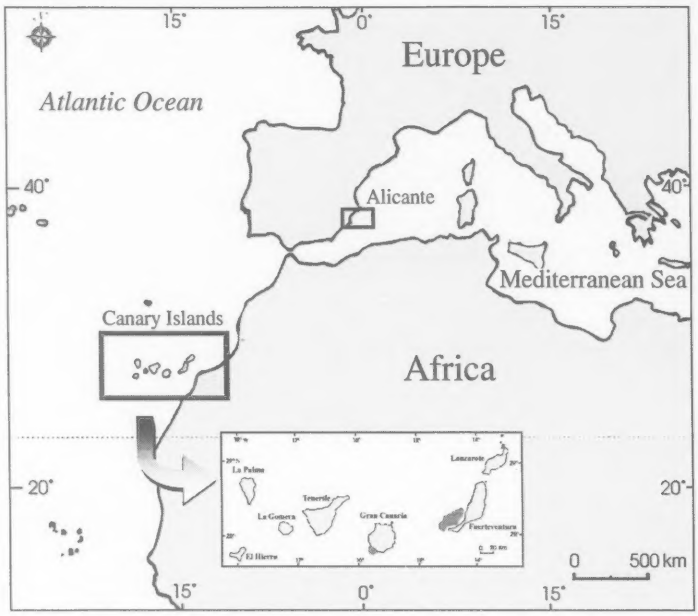

Figure 1

Location of sampling areas (shown with dark shading) for blacktail comber (*Serranus atricauda*) specimens from the Canary Islands (eastern-central Atlantic).

Materials and methods

Sampling

A total of 425 individuals of *S. atricauda*, ranging from 15.7 to 43.2 cm total length were sampled monthly from commercial catches off the Canary Islands between September 1992 and November 1994 (Fig. 1).

Total length (TL), to the nearest centimeter, was measured for each specimen, together with the total and gutted weight (TW and GW, respectively), gonad weight (GNW), and liver weight (LW), with an accuracy of 0.1 g. Gonads were removed and fixed in 10% buffered formaldehyde. After 24–48 h, they were dehydrated, embedded in paraffin wax, sectioned in longitudinal or cross-sections (4 to 5 μm thick), and stained with Harris "hematoxylin-Puttis" eosin. Oocyte stages and spermatogenic cells were classified according to García-Díaz et al. (2002). Postovulatory follicles and atresia were also characterized according to the criteria used for *Engraulis mordax* by Hunter and Goldberg (1980) and Hunter and Macewicz (1985b). Maturity stages (MS) were determined from histological observations (the development of the ovary and testis and also the presence and absence of different types of the oocytes and spermatocytes) according to García-Díaz et al. (1997, 2002) (Table 1). The developmental stages of the oocytes were categorized according to Selman et al. (1993): primary growth (stage I); cortical alveoli formation (stage II); vitellogenesis (stage III); oocyte maturation (stage IV); and mature egg (stage V).

Seasonality of gonad development

Monthly changes in the five following variables were analyzed to determine the spawning season of blacktail comber (García-Díaz et al., 1997):

1 Percent frequency of the maturity stages; this indicates population changes in gonad development.
2 Gonadosomatic index (*GSI=(gonad weight/gutted weight)*×100): this shows differences in development of the gonads with respect to gutted body weight;

Table 1

Description of gonad stages according to García-Díaz et al. (1997, 2002).

Maturity stage	Histological appearance
I. Immature	Ovary contains oogonia and oocytes from primary growth (stage I). Testis formed mainly by spermatogonia and primary spermatocytes. Ovary and testis joined by connective tissue.
II. Developing virgin, or recovering-spent	Ovary begins to acquire ovarian lamellae. Contains stage-I oocytes and yolk-vesicle-formation-stage oocytes (stage II) in advanced phases. Testis arranged in tubules with spermatogonia and primary and secondary spermatocytes.
III. Developing, maturing	Ovary with oocytes at all previous stages and oocytes in vitellogenic stage (stage III). Seminiferous tubules contain all spermatogenic cells.
IV. Ripe	Ovary with oocytes at all previous stages and with maturation oocytes (stage IV), mature and hydrated eggs (stage V). Atresic oocytes and postovulatory follicles appear. Testis completely mature, tubules filled with spermatozoa (which accumulate in deferent duct next to gonadal wall) to be expelled.
V. Spent	Oocytes undergoing regression and reabsorption. Numerous atresic oocytes. Testis in regression; cells appear fused, form semicontinuous mass.

3 Hepatosomatic index ($HSI=(liver\ weight/gutted\ weight)\times 100$): this estimates the relative size of the liver to body weight:
4 Condition factor ($Kn=(total\ weight/\ TL^b)\times 1000$): this is as an overall measurement of robustness of the fish, b being the exponential of the regression $TW = aTL^b$, which is 3.25 according to Tuset et al. (2004);
5 Oocyte diameter (DO): this gives information on the cell development of each individual. To obtain this value, of ten fish randomly chosen from the monthly samples, the diameters of the first 50 oocytes encountered were measured with an ocular micrometer. Measurements were taken only of oocytes sectioned through the nucleus.

Length at sexual maturity

Total length of all individuals was used to estimate the size at first maturity and size at mass maturity. These are defined as the sizes (TL) at which 50% ($TL_{50\%}$) and 95% ($TL_{95\%}$) of all fish sampled are at the relevant maturity stage (developing MS III, ripe MS IV, or spent MS V) (García-Díaz et al., 1997). The proportions were estimated at length classes of 1 cm, and the data were fitted to the logistic curve (Pope et al., 1983):

$$p = 100/\left(1+\exp(a+bTL)\right),$$

where p = percentage of mature individuals as a function of size class (TL); and
a and b = specific parameters that can change during the life cycle.

A logarithmic transformation was applied to the equation to calculate the parameters a and b by means of linear regression.

Reproductive potential

The pattern of annual fecundity (indeterminate or determinate) was assessed by oocyte size-frequency distribution (Hunter and Macewicz, 1985a). Ten fish ranging between 18.1 and 31.5 cm TL in ripe stage were selected for analysis of oocyte size-frequency distribution—five fish in March and another five in July—to represent early and late gonad development (White et al., 2003). Because the mean size (t-test, $P>0.05$) was not significantly different between both samples, one frequency distribution was obtained for each month. The diameters of the first 500 oocytes encountered were measured with an ocular micrometer for each fish. Measurements were taken only of oocytes sectioned through the nucleus.

Gonads of individuals in MS III (developing) and MS IV (ripe) were collected to estimate fecundity. One lobule was fixed in 10% buffered formaldehyde (24 h) for histological study, and the other was weighed and preserved in Gilson's fluid to estimate fecundity. Gonads with postovulatory follicles were omitted from batch fecundity estimates (Hunter et al., 1985).

The oocyte size-frequency method was applied to determine the batch fecundity (Hunter et al., 1985). This entails counting the number of oocytes in the most developed modal group of oocytes and plotting the size-frequency. In each lobule 200 oocytes were measured with the Nikon digital counter SC-112 and data processor DP-201 connected to the micrometer stage of a profile projector (V-12A, Nikon, Melville, NY). The routine NORMSEP (normal distribution separator) of FAO-ICLARM Stock Assessment Tools (FISAT II, vers. 1.0.0, FAO, Rome, Italy) program was used to separate the most developed modal group of oocytes based on Hasselblad's maximum likelihood method (Hasselblad, 1966).

Batch fecundity was estimated gravimetrically by using the oocyte size-frequency method (Hunter et

al., 1985; Yoneda et al., 2001). Samples were filtered through a 100-mm mesh sieve (approximately the minimum diameter of vitellogenic oocytes, García-Díaz et al., 2002) and were carefully washed under running distilled water to eliminate tissue remains and fixer. We used a sieve made from a piece of nylon plankton net inserted between two sections of PVC pipe, 15 cm in diameter and 10 cm in depth (Lowerre-Barbieri and Barbieri, 1993). A compact mass of oocytes was dried for 24 hours on filter paper and weighed precisely to 0.001 g. Afterwards, a subsample of 0.2 g was selected and placed in the count dish, covered with 3–4 drops of glycerin, and the number of oocytes in the sample was tallied with a hand counter. Batch fecundity was considered to be the total number of oocytes within the most developed modal group of oocytes (Hunter et al., 1985).

Spawning frequency (the number of spawnings per year by an individual) was estimated by dividing the duration of the spawning season by the average number of days between spawning for all individuals (Hunter and Macewicz, 1985a). The duration of the spawning season was the number of days between the first (7th February) and last (16th July) occurrence of hydrated oocyte or postovulatory follicles. The average number of days between spawning was the inverse of the percent frequency of hydrated individuals, multiplied by 100 (Collins et al., 1998).

The potential annual fecundity estimates (PAFEs) were obtained by multiplying batch fecundity by spawning frequency, and relative fecundity was defined as PAFE divided by individual weight (Hunter et al., 1992). The relationships between PAFE and TL, PAFE and TW, and PAFE and gonad weight (GW) were calculated by the following compound equation:

$$PAFE = a\,(b^X),$$

where X = TL, TW, or GW; and
a and b = specific parameters.

A logarithmic transformation was applied to the equation to calculate parameters a and b by using linear regression (Zar, 1996).

Results

Spawning season and maturity sizes

The specimens revealed the presence of maturing and ripe individuals between December and October, excluding January because only one individual was sampled then. This finding may indicate that the population spawns throughout this period, peaking between February (37.5%) and June (89.8%) (Fig. 2).

The highest values for GSI (Fig. 3A) occurred between March (2.06) and July (3.37) and a peak of maximum activity occurred in June (3.93), decreasing over the following months. The HSI (Fig. 3B) and Kn (Fig. 3C) presented irregular values during the annual cycle. Consequently, no correlation was observed between either ovarian and liver growth with ovarian and fish growth. Oocyte diameter (DO) (Fig. 3D) values varied similarly to those from the GSI; highest values occurred from March (261.37 μm) to July (275.07 μm) and peaked in June (365.85 μm). However, the variability of oocyte diameter showed the presence of mature individuals (MS III + IV) in the months of September, October, and December, according to gonad classification.

The smallest individual with mature gonads was 16 cm TL. The maturity curve established a $TL_{50\%}$ of 19.3 cm and $TL_{95\%}$ of 33.1 cm (Fig. 4).

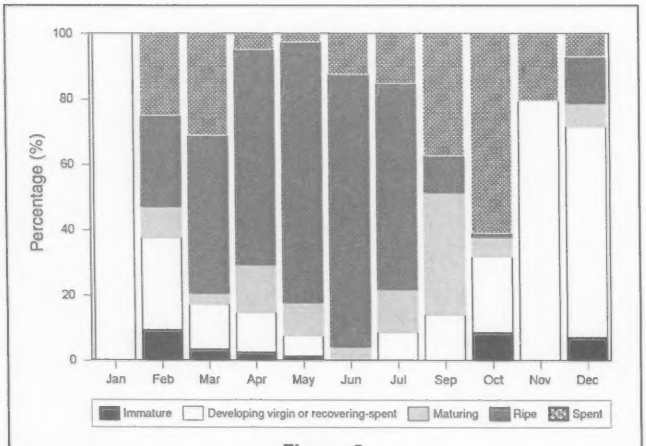

Figure 2

Monthly variation (%) in the maturity stages in the blacktail comber (*Serranus atricauda*).

Reproductive potential

From the oocyte size-frequency distributions, we concluded that the type of fecundity in this species is indeterminate evidenced by the lack of hiatus between advanced yolked oocytes and less mature oocytes (Fig. 5).

Batch fecundity estimates (n=28) varied between 21,774 and 369,578 oocytes. These estimates came from blacktail comber ranging in total length from 22.2 to 39.8 cm, total weight from 127.2 to 896.6 g, gutted weight from 122.9 to 834.6 g, and gonadal weight from 1.3 to 41.3 g.

The spawning-frequency estimate for individuals from 17.2 to 43.2 cm TL was 42 times/year, and 26.5% (45/170) of individuals spawned at an average of every 3.8 days.

Potential annual fecundity estimates ranged from 0.91 to 15.5 million oocytes, and an average of 5.1 ±4.1 million (Fig. 6). Relative fecundity varied between 5062 and 20,869 oocytes per gram of individual, and mean relative fecundity was 10,547 ±4148 oocytes.

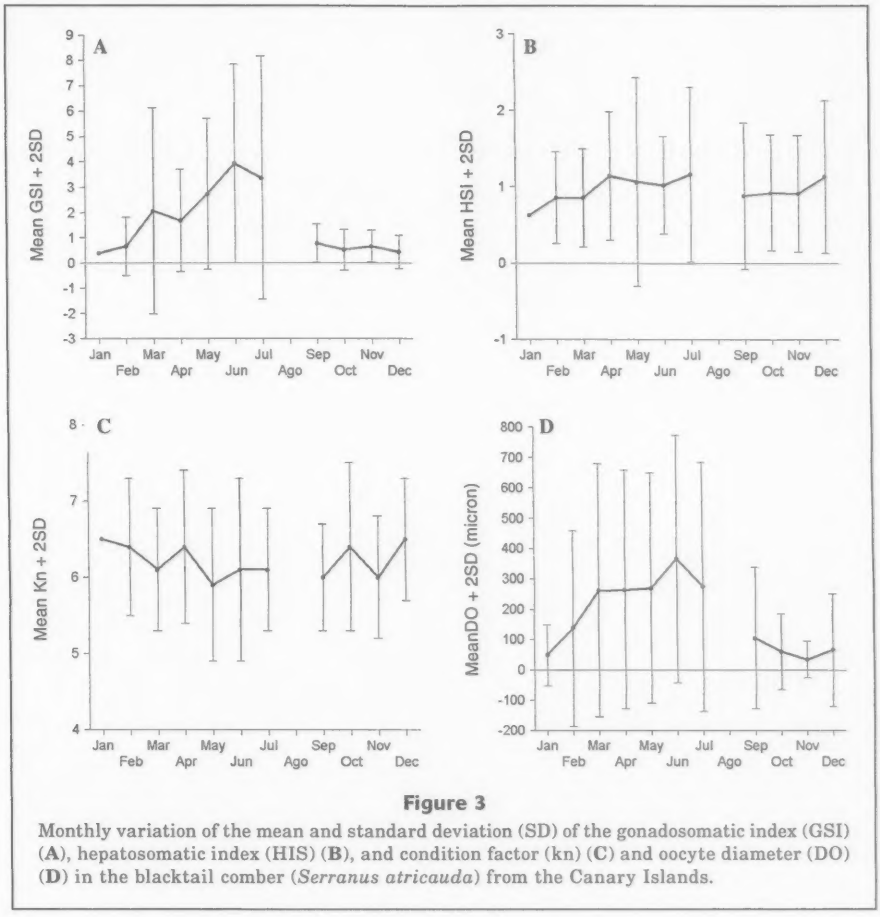

Figure 3

Monthly variation of the mean and standard deviation (SD) of the gonadosomatic index (GSI) (**A**), hepatosomatic index (HIS) (**B**), and condition factor (kn) (**C**) and oocyte diameter (DO) (**D**) in the blacktail comber (*Serranus atricauda*) from the Canary Islands.

Regression analysis showed significant positive correlation between annual fecundity and total length, total weight, and gutted weight. Total weight was the best predictor of annual fecundity ($PAFE$=1,053,504.23×1.0 0^{TW}; r^2=0.764, P<0.01, n=28); followed by total length ($PAFE$=63,648×4351×1380^{TL}; r^2=0.752, P<0.01, n=28); and gutted weight ($PAFE$=1,055,580×1.00^{GW}; r^2=0.723, P<0.01, n=28) (Fig. 6, A–C).

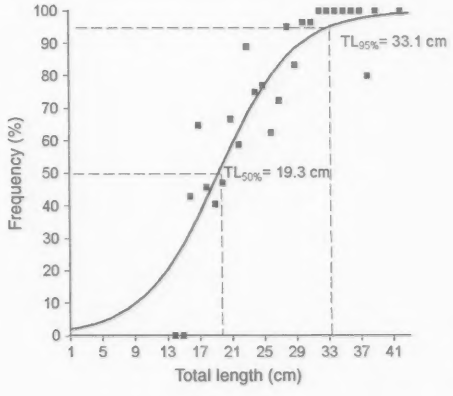

Figure 4

Sexual maturity curve and sizes at maturity for blacktail comber (*Serraus atricauda*) from the Canary Islands.

Discussion

The histological description of gonad structure is fundamental to understanding reproduction. In most reproductive fish studies the histological technique is omitted because it is too expensive and time-consuming. Consequently, in many cases knowledge of reproduction is limited or biased (or is both) (García-Díaz et al., 2001).

The spawning period in teleosts is determined from changes occurring within the gonad throughout the year. The macroscopic or histological observations of the

gonad (qualitative methods), somatic indices (e.g., GSI, HIS, and Kn), or evolution of oocyte diameter (quantitative methods) (or all four methods) are commonly applied. Many authors have disagreed about their applications and biological interpretation (De Vlaming et al., 1982; Wootton, 1990; Shapiro et al., 1993; Sadovy, 1996; Karlou-Riga and Economidis, 1997). Our results demonstrate that the GSI indicates only the spawning peak and not the presence of batch or multiple spawning within the species; and the HSI and Kn do not exhibit a clear trend throughout the year. Normally, variations of these indices (HSI and Kn) imply energy storage for reproduction (Hoar et al., 1983; N'Da and Déniel, 1993). However, *S. atricauda* does not require such storage because feeding activity is not altered during the reproductive period (Morato et al., 2000). Oocyte diameter presents a trend similar to that of GSI, although its biological interpretation is different. The oocyte may begin the vitellogenesis phase and there may be no significant increase in gonad weight. Consequently, GSI values do not necessarily change significantly when the spawning period has begun. Thus, the quantitative indices (GSI, HSI, Kn) show the general trend of the population, whereas the oocyte diameter provides information about the population as a whole, as well as at the individual level.

The analysis of qualitative and quantitative data in *S. atricauda* seems to indicate that spawning takes place throughout the year, although the general population spawns between March and July. In the Canary Islands, other members of the genus spawn over long periods: eight months in *S. cabrilla* and nine months in *S. scriba* (García-Díaz et al., 1997; García-Díaz 2003). These three species have the longest spawning season of all fish studied in the Canaries, although they are also the only species whose reproductive patterns have been analyzed with histological procedures. Nevertheless, environmental or biological factors (or both) must be related to this extended spawning period.

To estimate spawning frequency, the hydrated oocyte method is less time consuming but the POF method is better because the spatial and temporal distribution of the postovulatory follicle structures is not continuous (Hunter and Goldberg, 1980). Reproductive potential measured as potential annual fecundity has not been addressed in any species of the genus *Serranus* with the POF method to date. It is true that this fecundity must be considered as a rough estimation because the spawning frequency was a preliminary estimation because of the scarce number of individuals in sampling months. Siau and Bouian (1994) calculated the total fecundity in *S. cabrilla* and *S. scriba,* considering all the vitellogenesis oocytes. Their method has been widely rejected because it is valid only for

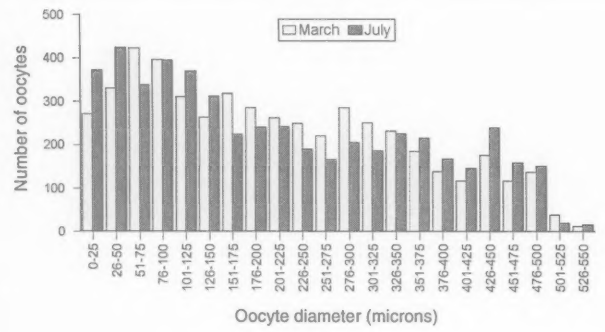

Figure 5

Oocyte size-frequency distributions (25-μm intervals) for blacktail comber (*Serraus atricauda*) ovaries from the Canary Islands.

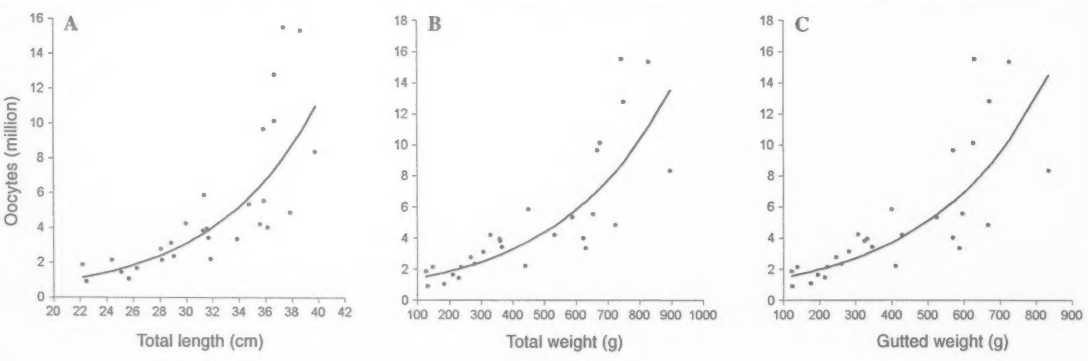

Figure 6

Relationships between potential annual fecundity and total length (**A**), total weight (**B**), and gutted weight (**C**) for blacktail comber (*Serranus atricauda*) from the Canary Islands.

determinate spawners, and both the above-mentioned serranids are indeterminate spawners (García-Díaz et al., 1997; García-Díaz, 2003). The fact that the method was used for these two indeterminate spawners could explain why the values of maximum fecundity obtained by these authors for *S. cabrilla* (441,502 oocytes) and *S. scriba* (54,649 oocytes) were lower than that for *S. atricauda* (15,5 millions oocytes). Consequently, the present study enables us to provide new reproductive data for blacktail comber, where potential annual fecundity is essential for future egg production models and estimates of spawning stock biomass.

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Benthic invertebrates that form habitat on deep banks off southern California, with special reference to deep sea coral

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Abstract—There is increasing interest in the potential impacts that fishing activities have on megafaunal benthic invertebrates occurring in continental shelf and slope ecosystems. We examined how the structure, size, and high-density aggregations of invertebrates provided structural relief for fishes in continental shelf and slope ecosystems off southern California. We made 112 dives in a submersible at 32–320 m water depth, surveying a variety of habitats from high-relief rock to flat sand and mud. Using quantitative video transect methods, we made 12,360 observations of 15 structure-forming invertebrate taxa and 521,898 individuals. We estimated size and incidence of epizoic animals on 9105 sponges, black corals, and gorgonians. Size variation among structure-forming invertebrates was significant and 90% of the individuals were <0.5 m high. Less than 1% of the observations of organisms actually sheltering in or located on invertebrates involved fishes. From the analysis of spatial associations between fishes and large invertebrates, six of 108 fish species were found more often adjacent to invertebrate colonies than the number of fish predicted by the fish-density data from transects. This finding indicates that there may be spatial associations that do not necessarily include physical contact with the sponges and corals. However, the median distances between these six fish species and the invertebrates were not particularly small (1.0–5.5 m). Thus, it is likely that these fishes and invertebrates are present together in the same habitats but that there is not necessarily a functional relationship between these groups of organisms. Regardless of their associations with fishes, these invertebrates provide structure and diversity for continental shelf ecosystems off southern California and certainly deserve the attention of scientists undertaking future conservation efforts.

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Science and conservation communities are increasingly interested in the potential impacts that fishing activities have on megafaunal benthic invertebrates found in continental shelf and slope ecosystems (Dayton et al., 2002; NRC, 2002; Malakoff, 2004; Roberts and Hirshfield, 2004; Rogers, 2004). Megafaunal invertebrates (>5 cm in height) contribute significantly to biodiversity, play important functional ecological roles, and can be indicators of long-term environmental conditions (e.g., Riedl, 1971; Palumbi, 1986; Brusca and Brusca, 1990). Moreover, because large invertebrates, such as sponges and corals, enhance the diversity and structural component of fish habitat and are vulnerable to impacts by at least some fisheries, they also may signify habitat areas of particular concern (HAPC) and as such would be protected under the Magnuson-Stevens Fishery Conservation and Management Act (Freese, 2001; Etnoyer and Morgan[1]).

[1] Etnoyer, P., and L. Morgan. 2003. Occurrences of habitat-forming deep sea corals in the Northeast Pacific Ocean. Technical Report, NOAA Office of Habitat Conservation, 31 p. Marine Biology Conservation Institute, 15806 NE 47th Ct., Redmond, WA 98052.

Deep-sea corals, such as gorgonians (sea fans), antipaththarians (black corals), scleractinians (stony corals), and hydrocorals, are of particular interest because they are often long-lived and slow-growing (Andrews et al., 2002; Heifetz, 2002), poorly studied (Etnoyer and Morgan[1]), and in certain situations vulnerable to human activities (e.g., mobile fishing gear) (Watling and Norse 1998; Freese et al., 1999; Krieger, 2001; Dayton et al., 2002; Fossa et al., 2002; NRC, 2002;). Other megafaunal invertebrates, such as crinoids, basket stars, and sponges also may enhance the structural components of fish habitat (Puniwai, 2002) and may be disturbed or destroyed by some fishing activities (Freese, 2001; Krieger, 2001).

The potential for invertebrates to add functional structure to benthic communities has centered largely around their size and complex morphology. A size threshold of 1 m has often been used as an indicator of structure-forming species because marked changes in benthic community structure have been observed in areas where rocky substrata exceed 1 m (Lissner and Benech, 1993). The complex structure of deep-sea corals also has been discussed as an important factor that contributes to microhabitat diversity (Krieger and Wing, 2002; Etnoyer and Morgan[1]). In this article, besides forming complex structure and large size, we also believe that megafaunal invertebrates form structure if they aggregate in high numbers, especially in areas of low relief. For example, aggregations of sea urchins and sea pens may provide significant structural relief for fishes in mud- and sand-dominated habitats (Brodeur, 2001).

An important question is the extent to which structure-forming invertebrates are ecologically important to fishes, especially those of economical value. Most studies have focused on "associations" between structure-forming invertebrates and fishes as a measure of ecological importance at several spatial scales. Fishes have been considered to be associated with invertebrates if they are found in the same trawl sample (Heifetz, 2002), if fishing is higher in areas with corals than without corals (Husebo et al., 2002), if they are found together within similar habitats observed from a submersible (Hixon et al.[2]), or if they are observed "among or within 1 m" from corals (Krieger and Wing, 2002). In this article we investigated association at three different levels: 1) fishes that are physically touching large invertebrates; 2) fishes that are found statistically more frequently near large invertebrates in relation to their overall abundance patterns; and 3) fishes that are found as nearest neighbors to large invertebrates.

The goal of this study was to describe patterns in the density, distribution, and size of structure-forming megafaunal invertebrates on deep rocky banks and outcrops off southern California. Given the recent interest in these organisms as potentially important habitat for groundfishes, and thus targets for protection from fishing activities, these organisms deserve a critical examination of their potential to contribute structure to continental shelf and slope ecosystems and an examination of their associations with fishes and other marine organisms. Accordingly, our specific objectives were the following:

- Identify structure-forming invertebrates based on criteria of size, morphological complexity, and density;
- Quantify the density and size distributions of these invertebrates according to depth and substratum types;
- Quantify associations between large, structure-forming invertebrates and other organisms, particularly fishes; and
- Assess the health of these organisms in terms of obvious physical damage.

Materials and methods

Underwater surveys were conducted off southern California by using nonextractive video-transect methods and direct observations from an occupied research submersible (*Delta*) from 8 October to 6 November 2002. These surveys were conducted as part of a larger investigation into the abundance, size, and distribution of cowcod (*Sebastes levis*) and associated benthic fishes and habitats inside and around the newly established Cowcod Conservation Areas (CCAs) off southern California (Fig. 1). The CCAs, which encompass 14,750 km^2 and are closed to groundfish harvest in water depth >37 m, were established in 2001 to assist in rebuilding the depleted cowcod population off southern California.

Digital, georeferenced maps of seafloor substratum types, interpreted from side-scan sonar, multibeam bathymetry, seismic reflection, and other past geophysical surveys, were used to identify and select sites of rocky habitats (Greene et al.[3]). We attempted to restrict the substratum types to mixed sediment and rock and to 30–330 m depth (i.e., likely cowcod habitat).

The *Delta* submersible was tracked by using an ORE Trackpoint II plus (ORE Offshore, West Wareham, MA) USBL system and WINFROG (vers. 3.1, FUGRO, San Diego, CA) software. We linked the tracking system to our ArcView® GIS (vers. 3.2, ESRI Corp., Redlands, CA) seafloor mapping project and tracked the submersible real-time in relationship to depth and seafloor habitat maps.

[2] Hixon, M. A., B. N. Tissot, and W. G. Pearcy. 1991. Fish assemblages of rocky banks of the Pacific northwest, Heceta, Coquille, and Daisy Banks. OCS Study MMS 91-0052, 410 p. U.S.D.I. Minerals Management Service 770 Paseo Camarillo, 2nd Floor, Camarillo, CA 93010.

[3] Greene, H. G., J. J. Bizzarro, D. M. Erdey, H. Lopez, L. Murai, S Watt, and J. Tilden. 2003. Essential fish habitat characterization and mapping of California continental margin. Moss Landing Marine Laboratories Technical Publication Series No. 2003-01, 29 p., 2 CDs. Moss Landing Marine Laboratories, 8272 Moss Landing Rd., Moss Landing, CA 95039.

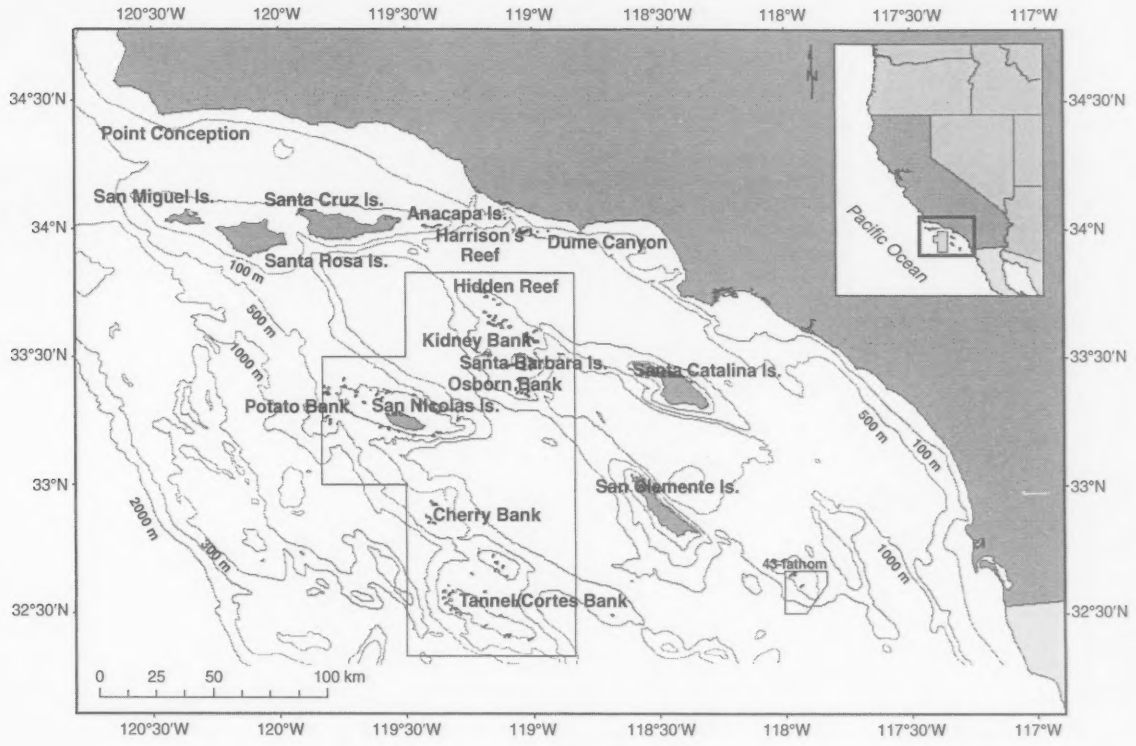

Figure 1

Locations of survey sites and dives (black dots) conducted inside and outside the cowcod conservation areas (delineated by outlined boxes) off southern California in 2002 to ascertain patterns in the density, distribution, and size of habitat-forming invertebrates.

We documented dives continuously during daytime hours with an externally mounted high-8 video camera positioned above the middle viewing-porthole on the starboard side of the submersible. The observer verbally annotated all tapes with observations on fishes, invertebrates, and physical habitats. Two parallel lasers were installed 20 cm apart on either side of the external video camera for estimating fish and invertebrate sizes and delineating a 2 m-wide belt transect for counting fishes and invertebrates. We used personal dive sonar from inside the submersible to verify the width of swath for the belt transects. Digital still and video cameras were used inside the submersible to help document fishes, invertebrates, and habitats.

We defined "habitat" using a combination of nine different categories of substratum and standard geological definitions (see Stein et al., 1992; Yoklavich et al., 2000). In order of increasing particle size or relief, these substrata were the following: mud (code M), sand (S), gravel (G), pebble (P), cobble (C), boulder (B), continuous flat rock (F), rock ridge (R), and pinnacles (T). A two-character code was assigned each time a distinct change in substratum type was noted along the transect, thus delineating habitat patches of uniform type. The first character in the code represented the substratum that accounted for at least 50% of the patch, and the second character represented the substratum accounting for at least 20% of the patch (e.g., "BC" represented a patch with at least 50% cover by boulders and at least 20% cover by cobble). Each habitat patch also was assigned a code based on the degree of its three-dimensional structure as defined by the vertical relief of the physical substrata from the seafloor. Habitats were coded as 1=low (<1 m), 2=moderate (1–5 m), or 3=high relief (>5 m). Patches less than 10 seconds in duration were not recorded. The area of each habitat patch was determined by calculating the distance between the beginning and end of habitat patches with ArcGIS® and multiplying by the width of the transect (2 m).

A total of 58 different types of habitat patches were observed across all dives. These data were analyzed by a cluster analysis (Euclidean distance, group average method) by using the abundances of the 20 most common invertebrate species, and the resulting dendrogram was used to pool the number of codes into the 17 most distinct habitat types exhibiting a similarity of >50%.

Direct counts of megafaunal invertebrates were made from videotapes within each habitat patch; patch areas varied from 12–1472 m^2. Densities of invertebrates were estimated by dividing the total number of individuals

Table 1

Number of submersible dives and habitat patches surveyed for structure-forming invertebrates on rocky outcrops inside and outside the cowcod conservation areas in the southern California borderland.

Study site	No. of dives	No. of habitat patches
Inside the cowcod conservation areas		
43-fathom	4	142
Cherry Bank	8	266
Hidden Reef	5	156
Kidney Bank	21	474
Osborn Bank	5	199
Potato Bank	4	113
San Nicolas Island	23	785
Santa Barbara Island	9	203
Tanner and Cortes banks	21	587
Outside cowcod conservation areas		
Harrison's Reef	9	198
Dume Canyon	3	66
Total	112	3189

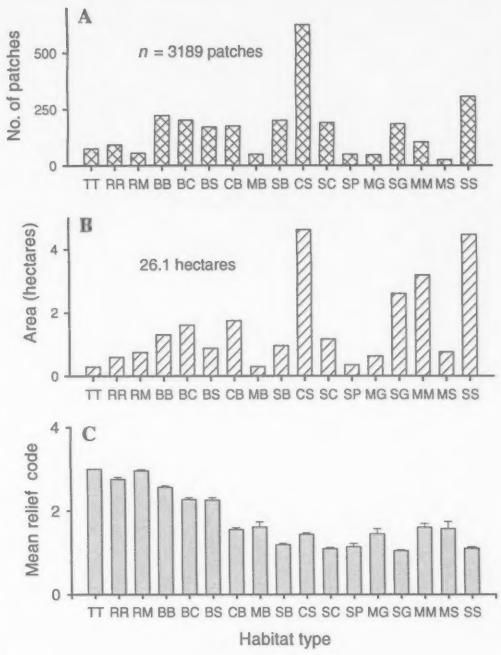

Figure 2

Characteristics of habitat patches surveyed on southern California rocky banks. (**A**) Number of patches in each substratum type. (**B**) Total area of each substratum type. (**C**) Mean relief (±1 standard error). See text for description of method and habitat codes. See page 169 for definitions for the substrate abbreviations along the *x* axis.

in each group, identified to the lowest taxonomic level, by the area of their associated habitat patches. For the larger invertebrates we recorded the geographic position and estimated maximum height of each solitary sponge, gorgonian, and black coral. We also noted the color of black corals. We made observations on the occurrence of animals (i.e., epizoids) found directly on sponges, gorgonians, and black corals, and also noted any damaged or dead individuals. Voucher specimens were collected to assist in taxonomic identification.

To quantify fish-invertebrate associations we used ArcGIS® to estimate the distance between each sponge, gorgonian, and black coral invertebrate and the nearest fish. These data were compared to the total number of fishes counted in habitats that contained sponges, gorgonians, and black corals by using a chi-square test to look for significant differences in the frequency of fish observed near corals in relation to overall fish abundance.

Results

We completed 112 dives and surveyed 3189 habitat patches (Table 1), covering 26.1 hectares at 32–320 m depths (median depth of 110 m). The distributions of number of patches and of surface area of habitats were similar except for sand (SS), sand-gravel (SG), and mud (MM) habitats, all of which had greater surface areas in relation to number of patches (Fig. 2, A and B). Overall, cobble-sand (CS), sand, mud, and sand-gravel constituted the largest habitat areas, but cobble-sand and sand were the most frequent habitat types. Vertical physical structure varied among habitat types; the highest structure was found in high-relief rock areas (TT to BS) and lower structure was found in low-relief mixed rock (CB to SP) and mixed sediment areas (MG to SS; Fig. 2C). The frequency of patches of each substratum type varied by depth (Fig. 3). The incidence of most high-relief rock categories (TT, RR, BB, and BS) decreased with depth, and the occurrence of mud-dominated habitat patches (MB, MG, MS, MM) increased with depth.

Overall, 12,360 observations were made on 521,898 individuals from 15 taxa of megafaunal, structure-forming invertebrates; during these observations, estimates of size and incidence of epizoic animals on 9105 sponges, black corals, and gorgonians were made (Table 2). The most common structure-forming invertebrates (98% of total) included the crinoid *Florometra serratissima* (40%), the brittle star (*Ophiacantha* spp. [33%]), brachiopods (order Terebratulida [11%]), the white sea urchin (*Lytechinus anamesus* [9%]), the fragile sea urchin

(*Allocentrotus fragilis* [4%]), and sea pens (suborder Subselliflorae; [2%]; Fig. 4).

The density of common structure-forming invertebrates was variable across habitat types; some species were found over a wide range of habitats. Crinoids and basket stars were found on all 17 habitat types but were most dense on either high-relief rock or low-relief mixed rock (Fig. 5). In contrast, brittle stars and brachiopods were dense in low-relief mixed rock but rare or absent in low-relief mixed sediment. White sea urchins were most dense in habitats with sand, whereas fragile sea urchins were most dense in habitats with mud. White-plumed anemones were most dense in mud-gravel habitats, and sea pens were most dense in low-relief mixed-sediments (Fig. 5).

Deep sea corals and sponges were the largest structure-forming invertebrates but were relatively uncommon (2% of total) (Table 2). Gorgonians were difficult to distinguish and were categorized into one group (order Gorgonacea). The black coral is a new species that recently has been described and named the Christmas tree coral (*Antipathes dendrochristos)* (Opresko, 2005). Sponges were categorized into five groups based on their structure and shape: flat, barrel, shelf, vase, and foliose sponges (Fig. 6).

Gorgonians and black corals were most dense on low-relief mixed rock areas (Fig. 7). However, gorgonians were found in only four habitat types at 144–163 m depth, whereas black corals were found on 12 habitat types at 100–225 m depth, including pinnacle, boulder, and sand areas. These differences may be due to the unequal number of observations (i.e., 27 gorgonian vs. 135 black coral colonies).

The five morphological groups of sponges displayed broad distributions across habitat types but were especially dense on high-relief rock and low-relief mixed rock (Fig. 7). Flat, barrel, vase, and foliose sponges were found in all habitats; shelf sponges were found in all habitats except MM, MS, and SS. Foliose sponges were found at significantly deeper depths (mean=191 m; SE=53; n=1259) than were other sponge groups (pooled mean=152 m; SE=0.6; n=7545), which were not significantly different from each other (Kruskal-Wallis H=594; df=4; P<0.01). Generally sponge size increased with increasing depth, although the correlation was low (r=0.07; P<0.001; n=6551). Although sponges were found throughout the study area, gorgonians and black corals were restricted in their distribution to a small number of sites (Fig. 8).

Structure-forming invertebrates displayed wide variation in size; maximum height ranged from 4 cm for brachiopods to 2.5 m for black corals (Table 2). There was no significant correlation between size of the invertebrate taxa and structural relief of the substratum types (r=0.28, P=0.30, n=15).

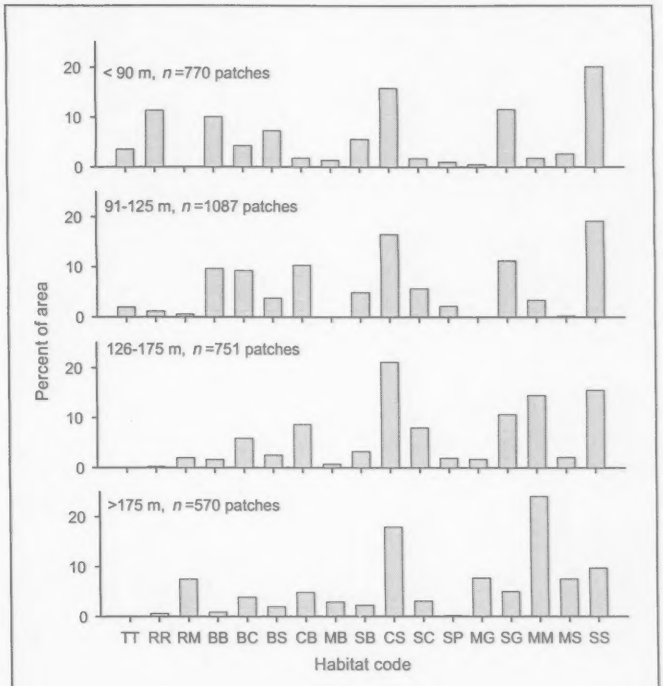

Figure 3

The frequency of habitat patches of each substratum type, stratified by depth. See page 169 for definitions for the substrate abbreviations along the x axis.

Gorgonians and black corals had different size distributions (Fig. 9). Black corals ranged from 10–250 cm in height (mean=33.6; SE=1.9; n=195) and most individuals were in the 10–50 cm size category. Individuals were found in three color forms: gray-to-white (50%), rusty-brown-to-red (47%), and gold (3%). Gorgonians ranged in size from 10 to 40 cm (mean=21.7; SE=1.2; n=27) and were found in multiple morphological forms from elongate to fan-like (Fig. 6).

Sponges displayed similar size distributions to those of gorgonian corals, but had different mean and maximum sizes (Fig. 9). Mean sizes of flat, barrel, and foliose sponges were not significantly different from each other (pooled mean=19.8 cm; SE=0.1; n=7373) but were significantly smaller than vase and shelf sponges (pooled mean=20.9 cm; SE=0.2; n=1289), which were not different from each other (one-way ANOVA; F=4.52; df=4,8657; P=0.001). The maximum observed height was 50 cm for shelf sponges, 60 cm for foliose sponges, and 100 cm for barrel, flat, and vase sponges (Fig. 9).

Most (98.2% by number) sponges, gorgonians, and black corals by number did not have any other organisms living on them (Table 3). Overall, crinoids (1.4%) were most commonly associated with these large invertebrates, followed by sponges (0.1%), and nine other

Table 2

Significant criteria of structure-forming invertebrates on rocky outcrops off southern California. "X" indicates taxa that exhibit large size, complex morphological shape, and high-density aggregations. SE is standard error and *n* is the total number of observations per taxon. The sample sizes for density and depth calculations were reduced because of incomplete observations on all individuals. Organisms are ordered from largest to smallest group.

		Criteria			Density (no./hectare)			Depth (m)		
Taxa	*n*	Size	Complex morphology	High density	Mean	SE	Maximum height (cm)	Mean	SE	Mean physical structural relief
Black coral (*Antipathes dendrochristos*)	135	X	X		10	4	250	183	24	1.7
Flat sponge	4240	X			312	53	100	149	43	1.9
Barrel sponge	2138	X			163	28	100	151	46	2.0
Vase sponge	1167	X			90	16	100	163	44	1.9
Sea pen (Subselliflorae)	9726	X		X	819	153	100	157	61	1.4
Basket star (*Gorgonocephalus eucnemis*)	733	X	X		58	12	90	162	44	1.7
White-plumed anemone (*Metridium farcimen*)	57	X			5	2	80	161	46	1.9
Foliose sponge	1259	X			96	25	60	191	53	2.0
Shelf sponge	139	X			10	3	50	158	47	2.1
Gorgonian (Gorgonacea)	27		X		3	3	40	159	4	1.2
Crinoid (*Florometra serratissima*)	174,231		X	X	22,045	8466	35	146	46	1.6
Brittle star (Ophiuridae)	207,667			X	17,786	4301	15	148	47	1.8
Fragile sea urchin (*Allocentrotus fragilis*)	18,363			X	2031	668	8	185	45	1.8
White sea urchin (*Lytechinus anamesus*)	45,092			X	4631	1388	4	85	20	1.2
Brachiopod (Order Terebratulida)	56,924			X	3623	1705	4	100	17	1.8

Table 3

Associations of organisms with large structure-forming invertebrates on rocky banks off southern California.

		Associated organisms (% of total observations)											
Taxa	*n*	None	Crinoids	Sponges	Fishes	Egg cases	Crabs	Basket stars	Brittle stars	Sea stars	Anemone	Algae	Salps
Foliose sponge	1262	99.0	0.6	0.3	0	0	0	0	0	0	0	0	0
Flat sponge	4043	99.5	0.5	<0.1	0	0	0	<0.1	0	0	0	0	0
Barrel sponge	2068	98.0	1.9	0	0	0	0	0	0.1	0.1	0	0	0
Shelf sponge	134	99.3	0.7	0	0	0	0	0	0	0	0	0	0
Vase sponge	1155	96.1	3.6	0	0.1	0.1	0	0.1	0	0	0	0	0
Gorgonian	27	100	0	0	0	0	0	0	0	0	0	0	0
Black coral	194	84.7	6.8	3.1	1.3	0	1.8	0.4	0.4	0	0.4	0.4	0.4
Total	8883	98.2	1.4	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

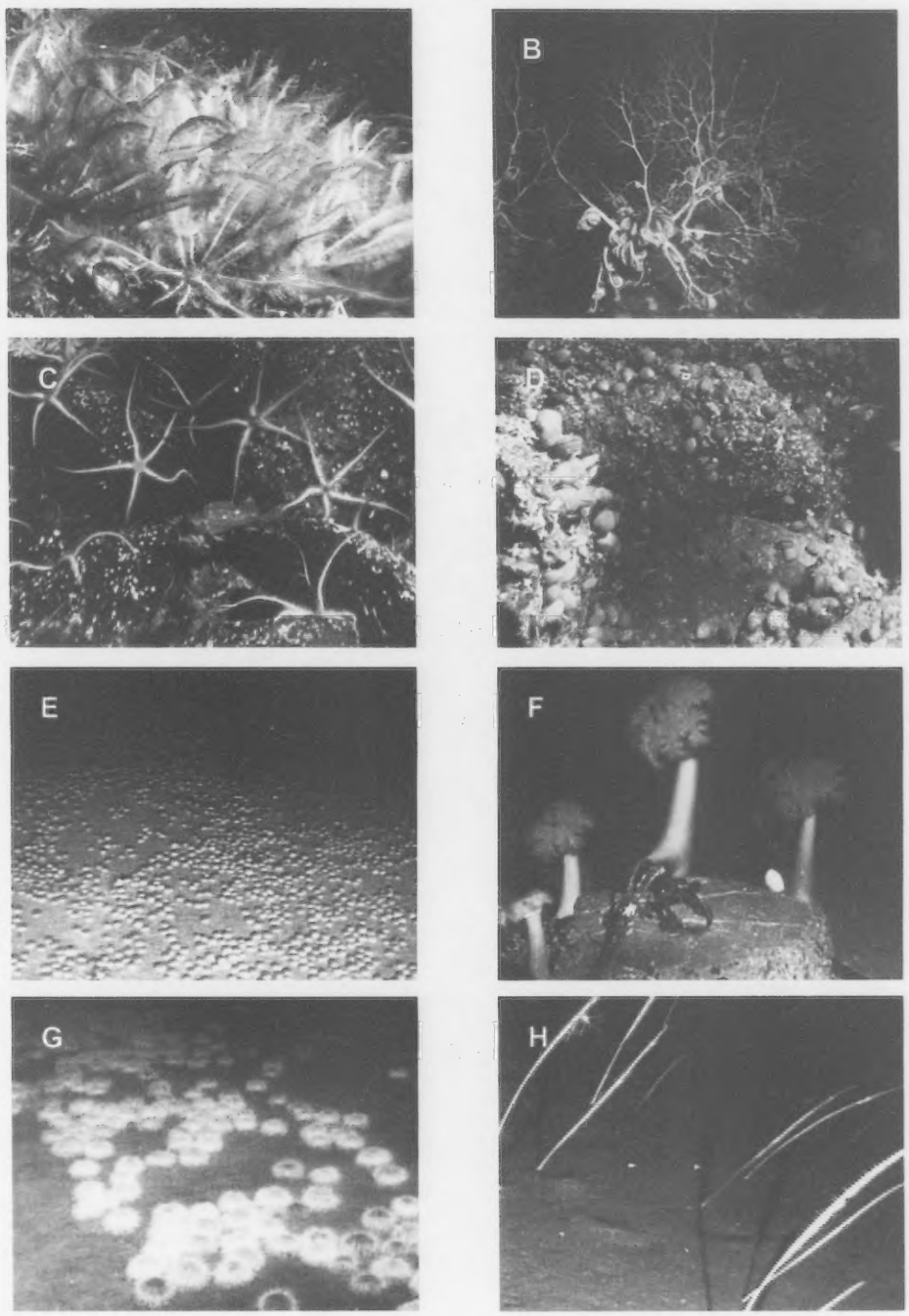

Figure 4

Some major structure-forming invertebrates on southern California rocky banks: (**A**) crinoids (*Florometra serratissima*), (**B**) basket stars (*Gorgonocephalus eucnemis*), (**C**) brittle stars (*Ophiacantha* spp.), (**D**) brachiopods (Terebratulida), (**E**) white sea urchins (*Lytechinus anamesus*), (**F**) white-plumed anemones (*Metridium farcimen*), (**G**) fragile sea urchins (*Allocentrotus fragilis*), and (**H**) sea pens (Subselliflorae).

taxa (all <0.1%). Black corals had the largest incidence of associated animals (15.3% of individuals), followed by vase (3.9%), barrel (2%), foliose (1%), shelf (0.7%), and flat (0.5%) sponges. Fish were most commonly observed on black corals (1.3%) but were also observed on vase sponges, including an attached egg case. No organisms were observed living on gorgonians.

The frequency of fish species near sponges, gorgonians, and black corals was significantly different from the frequency of those same species found elsewhere along transects (chi-square, all $P<0.01$; Table 4). Of the 108 species adjacent to these large invertebrates, six species were found at significantly higher frequencies than predicted by their density along transects: cowcod, bank rockfish (*Sebastes rufus*), swordspine rockfish (*Sebastes ensifer*), shortbelly rockfish (*Sebastes jordani*), pinkrose rockfish (*Sebastes simulator*), and members of the rockfish subgenus *Sebastomus* (Table 4).

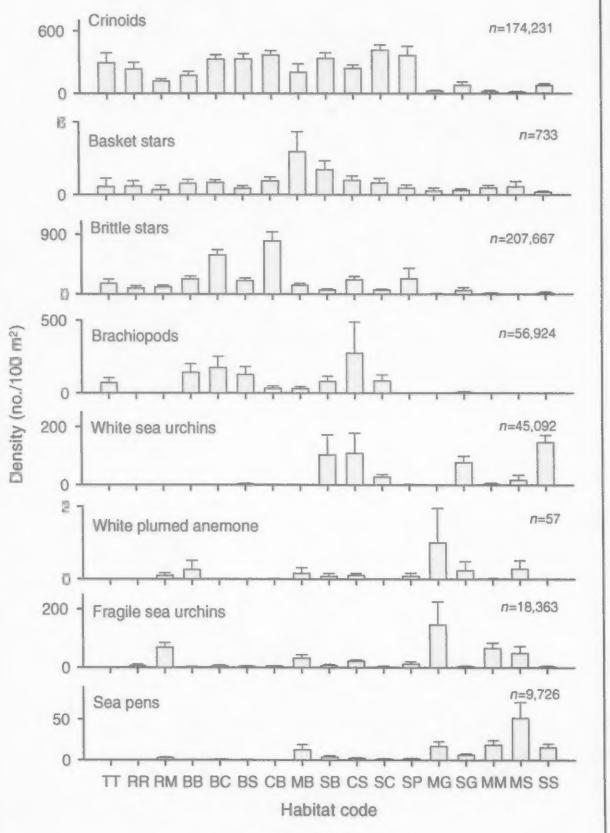

Figure 5

Mean density of structure-forming invertebrates in habitat patches of each substratum type. Vertical bars are ± one standard error. See page 169 for definitions for the substrate abbreviations along the x axis.

The distribution of mean nearest-neighbor distances between fishes and large invertebrates varied from 0.1 to 9.9 m (Fig. 10). Overall median distances varied from 0.9 m (shelf sponges) to 1.8 m (black corals). However, there were no statistical differences between the median distances for each group (Kruskal-Wallis, $H=10.4$; df=6; $P=0.11$). For the six fishes that were found more frequently near large invertebrates than on transects, the overall median distances to the invertebrates were 5.5 m (cowcod), 1.0 m (bank rockfish), 1.3 m (swordspine rockfish), 1.5 m (shortbelly rockfish), 1.7 m (pinkrose rockfish), and 1.4 m (*Sebastomus*).

The overall incidence of damaged and dead sponges, gorgonians, and black corals was low (0.3% of total number observed). Black corals were more commonly damaged (1.7%) or dead (1.1%), followed by vase sponges (0.6% and 0.1%, respectively), barrel sponges (0.5% and 0%), and foliose sponges (0% and 0.1%). No dead or damaged flat or shelf sponges or gorgonians were observed. Damage in black corals included portions of the colony that appeared discolored; dead black corals lacked polyps and were discolored. Among sponges, the most common damage was individuals that had broken from the substratum and were lying on their side or broken colonies.

Discussion

Several groups of invertebrates were distinguished by their large size, such as black corals, sponges, crinoids, basket stars, anemones, and sea pens. Organism size is an important aspect of structural habitat because it contributes to vertical relief and increases the availability of microhabitats. For example, yelloweye (*Sebastes ruberrimus*) rockfish may use the large gorgonian coral *Primnoa* as a vantage point to prey upon small fishes (Krieger and Wing, 2002). Size variation among structure-forming invertebrates was significant. Individual black corals, sea pens, and sponges greater than 1 m in height represented only 0.1% of all organisms, and 90% of the individuals were <0.5 m high.

Similarly, the complex structures of crinoids, gorgonians, black corals, and basket stars may increase the availability of microhabitats and create a high surface area for settlement or retention of other organisms. Fish egg cases have been observed attached to both gorgonians (Etnoyer and Morgan[1]) and vase sponges (present study, Table 3).

High-density aggregations have not been used as a criterion for defining structure-forming invertebrates, but there are several examples that illustrate the potential importance of these aggregations. High density "forests" of crinoids provide refuge and substrata for a wide variety of small fishes and invertebrates in rocky areas (Lissner and Benech, 1993; Puniwai, 2002).

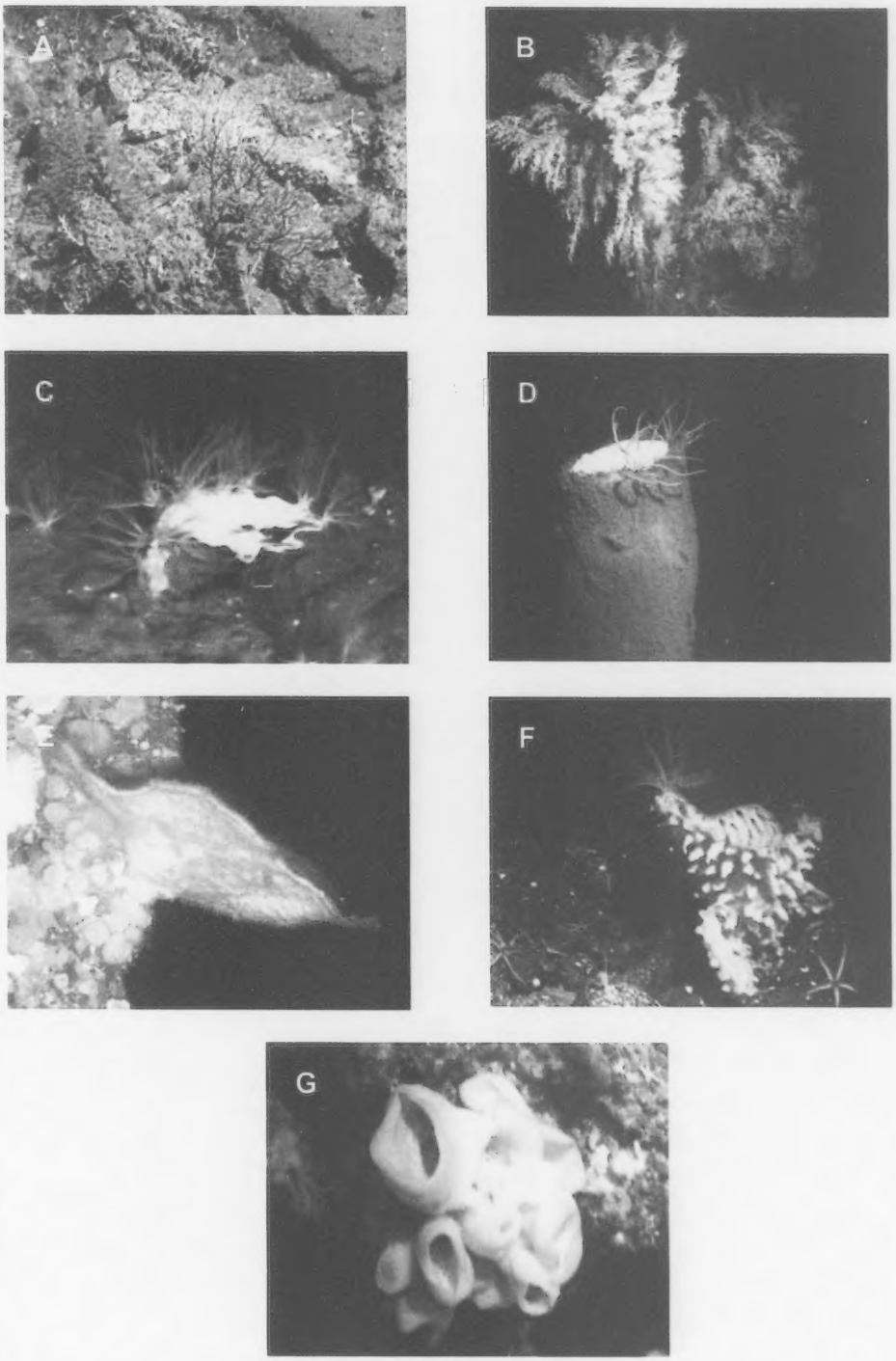

Figure 6

Structure-forming invertebrates: (**A**) gorgonians (Gorgonacea), (**B**) black coral (*Antipathes dendrochristos*), (**C**) flat, (**D**) barrel, (**E**) shelf, (**F**) vase, and (**G**) foliose sponges on southern California rocky banks.

Similarly, high-density aggregations of brittle stars and brachiopods in boulder-cobble areas and fields of sea pens and sea urchins in sand and mud habitats also may provide space and structure for other organisms (e.g., Brodeur, 2001).

With the exception of black corals and sea pens, the largest structure-forming invertebrates, such as sponges, *Metridium* spp., and crinoids, were most common on high- to moderate-relief rocky habitats. These long-lived organisms are likely to be favored in stable habitats that are more insulated from sediment transport and high particle loads than low-relief, mud-dominated areas (Lissner and Benech, 1993). Large invertebrates add structure and micro-scale complexity to these rocky habitats that already contain high-to-moderate amounts of relief. Sponges, with their broad distributions, may also provide structure for flatfishes in low-relief mud habitats (Ryer et al., 2004)

Black corals and gorgonians, in contrast, are more commonly found in current-swept areas near drop-offs and under ledges (Grigg, 1974; Parrish, 2004). In our study, these invertebrates were found in low-relief mixed cobble-boulder-sand habitats at 100–225 m depths, providing significant vertical structure for potential use by a wide variety of organisms.

Aggregations of sea pens and sea urchins may provide important structure in low-relief sand and mud habitats where there is little physical habitat complexity. In addition, these organisms may provide refuge for small planktonic and benthic invertebrates, which in turn may be preyed upon by fishes. They also may alter water current flow, thereby retaining nutrients and entraining plankton near the sediment. Urchins rapidly respond to patches of drift kelp (Harrold and Reed, 1985), which provide organic material to deep sea habitats (Harrold et al., 1998).

One of the central issues currently relevant to structure-forming invertebrates is the degree to which these species contribute to the spawning, breeding, feeding, or growth-to-maturity of economically important fishes. Although there are several studies that report fish-invertebrate associations within common habitats (Hixon et al.[2]), or make anecdotal or general observations on fish-invertebrate associations (e.g., Krieger and Wing, 2002), few studies have systemically quantified these relationships. In our study, for 9105 observations on the larger invertebrates found on southern California rocky banks only 1.8% of individuals had other organisms lying on or attached to them. Moreover, the vast majority of these organisms were other invertebrates, including crinoids, sponges, crabs, basket stars, brittle stars, seastars, anemones and salps. Less than 1% of the observations of organisms actually sheltering in or located on invertebrates involved fishes (a total of five individuals and one egg case), and most were observed on large black corals (Table 3). This result implies that fishes are not strongly associated with structure-forming invertebrates in the areas we surveyed off southern California.

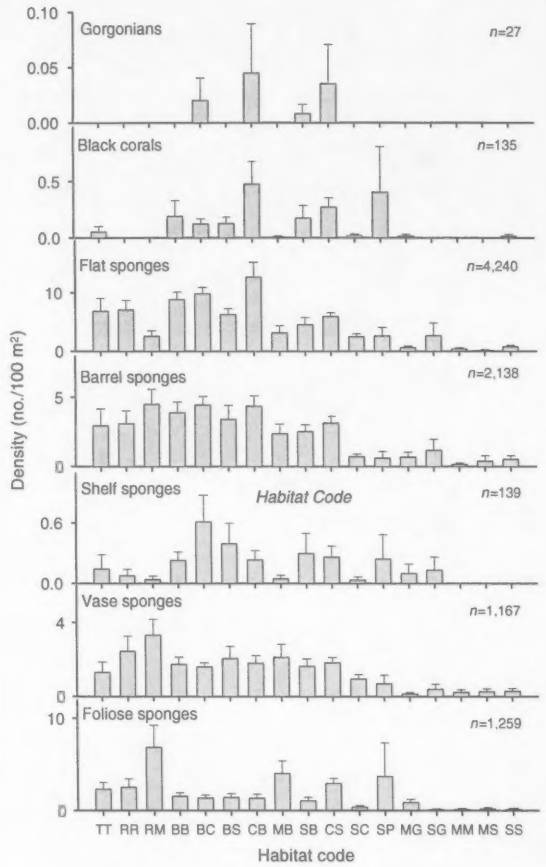

Figure 7

Mean density of gorgonians, black corals, and sponges in habitat patches of each substratum type. Vertical bars are ± one standard error. See page 169 for definitions for the substrate abbreviations along the *x* axis.

However, we should note that our observations were limited to daylight hours and that the viewing angle from the submersible generally precluded seeing inside some of the sponges (especially vase and barrel types). Moreover, our analyses focused on associations between fishes and individual solitary invertebrates, most of which were <0.5 m in height. We did not examine associations between all structure-forming invertebrates, nor did we examine associations between invertebrates and assemblages of fishes at the level of discrete habitat patches (100–1000 m scale) (e.g., Tissot et al.[4])

[4] Tissot, B. N., M. A. Hixon, D. L Stein. Unpubl. manuscript. Habitat-base submersible assessment of groundfish assemblages at Heceta Bank, Oregon from 1988–1990.

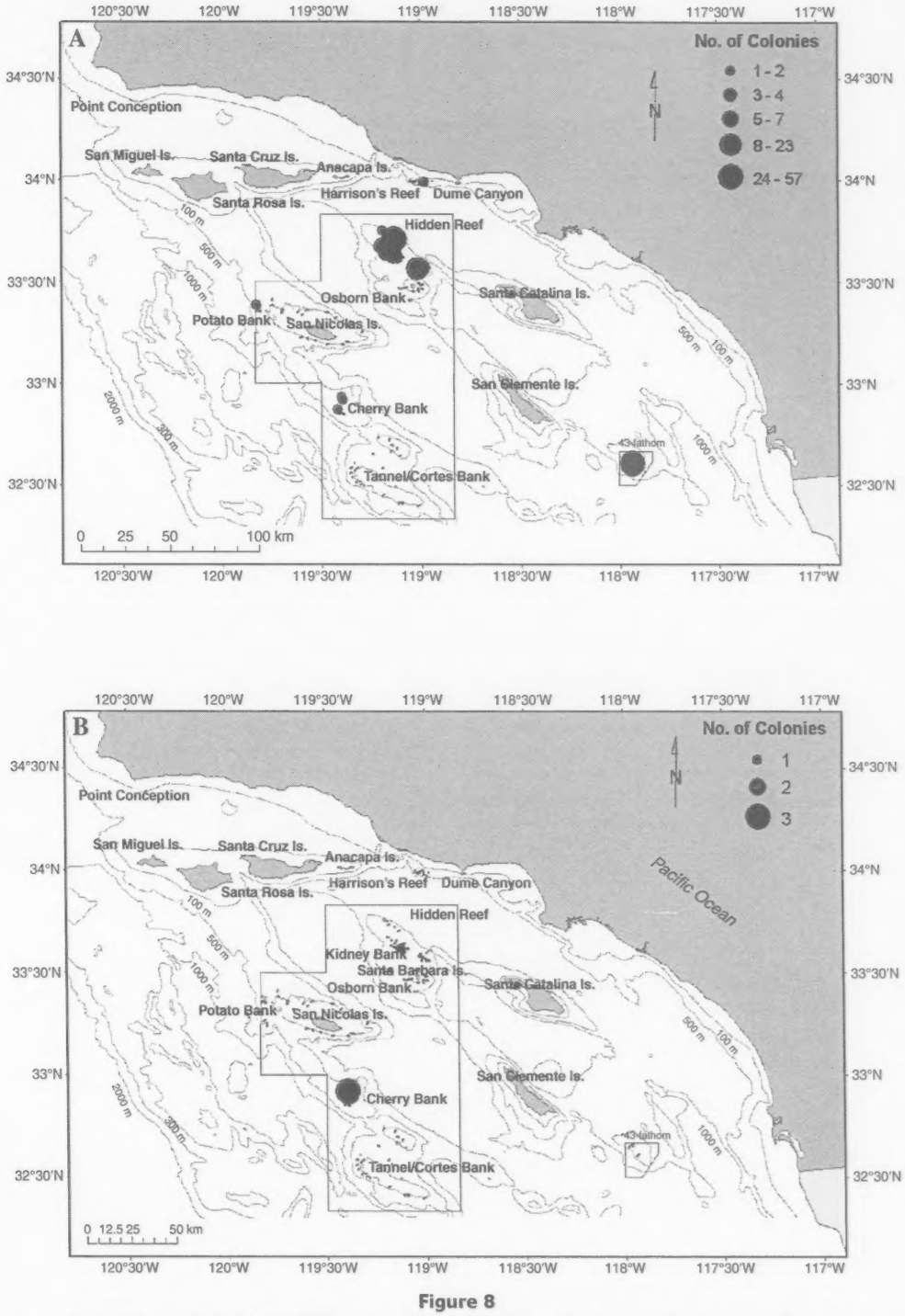

Figure 8

Distribution and number of (**A**) black coral and (**B**) gorgonian colonies inside and outside the cowcod conservation areas (delineated as outlined boxes) of southern California in 2002. Dive sites are indicated by black dots.

From the analysis of spatial associations between fishes and large, individual structure-forming invertebrates, six of 108 species were found more often adjacent to colonies than predicted by their abundance along transects. This result indicates that there may be spatial associations that do not necessarily include physical contact with the sponges and corals. However, the median distances between these six fish species and large invertebrates (1.0–5.5 m) were not particularly small. Thus, it is likely that these fishes and invertebrates are present in the same types of habitats and that there is not necessarily a functional relationship between these two groups of organisms.

Parrish (2004) reached similar conclusions on studies of black coral in Hawaii. Although fish densities were higher in areas that included corals, when bottom relief and depth were accounted for these densities were not higher than those for surrounding areas without corals. Thus, there was no clear evidence that corals served to aggregate fish. Rather, fishes and corals co-occurred in areas with similar physical relief and unique flow regime (Parrish, 2004). Auster (2005) also reached similar conclusions by finding no significant difference in the density of a common rockfish species (*Sebastes fasiatus*) between areas of rock and boulders with dense coral cover and similar areas having dense epifaunal cover (i.e., without coral). Auster concluded that although dense coral and dense epifaunal habitats were functionally equivalent, the epifaunal habitat was more widespread in his study area, making that habitat perhaps more important to the greater rockfish population. Finally, Syms and Jones (2001) demonstrated that removal of high densities of soft corals caused no significant changes in the associated fish communities and that the heterogeneity of habitat generated by soft corals was indistinguishable from equivalent habitat formed by rock alone. Thus, fish-invertebrate associations, by themselves, do not necessarily demonstrate the functional importance of invertebrates as habitat to benthic fish populations.

One possible conclusion from our study is that observed fish-invertebrate associations, like those reported for many cold-water corals, can be overstated. In the absence of quantitative information, observers may remember the few positive associations between fishes and structure-forming invertebrates but ignore (or forget) the more numerous observations of large invertebrates with no associated fishes. Indeed, the general impression of the authors after making submersible observations was that there were higher numbers of fishes associated with large invertebrates when in reality only five fishes were observed lying directly on a large invertebrate in the video transects. A more likely conclusion, however, is that the continental shelf communities of southern California are unique and that large black corals do not have the high number of commensals as seen, for example, on *Primnoa* in Alaska. An additional consideration is the relatively low number and size of individual sponges, gorgonians, and black corals observed in this study. *Primnoa* in Alaska can form massive stands 3 m tall and 7 m wide in some areas (Krieger and Wing, 2002). Moreover, the majority of fishes were observed on the largest individuals in their study (>15 m^3 in volume) Most of the corals in our study were <0.5 m in height. Given the importance of this issue, we argue for more rigorous quantitative studies on fish-invertebrate associations that would include densities of fishes and sizes of both fishes and invertebrates.

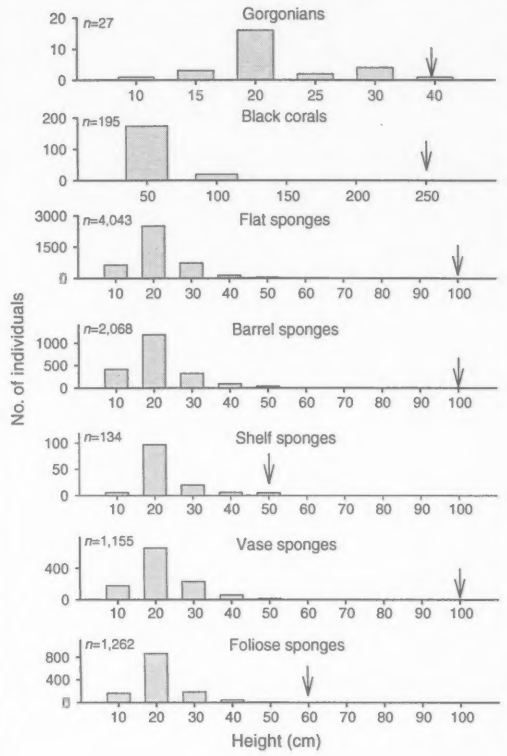

Figure 9

Size distributions of large, structure-forming invertebrates. Arrows indicate maximum sizes observed in each group.

Regardless of their associations with fishes, the structure-forming invertebrates described in this study are very likely to be ecologically important on continental shelf ecosystems and are certainly significant in their own right. Observation on the health of the larger invertebrates indicates few damaged (0.1%) or dead (0.2%) individuals and a low incidence of fishing gear in the areas surveyed (Tissot et al., unpubl, data). These observations are consistent with the absence of a significant commercial bottom trawling fishery in our survey area, which has been associated with negative impacts on large invertebrates in other locations (Watling and Norse, 1998; Freese et al. 1999; Krieger, 2001). Thus, this study affords a unique view of what appears to be

Table 4

Percentage of fish species near sponges, gorgonians, and black coral in relation to fishes counted along transects. Species that are less than 1% of the overall total number along all dives are not included. Species that were found more often near these invertebrates than expected by chance are indicated with an *.

	Common sponges					Shelf sponges		Gorgonians		Black coral	
Taxa	Total near tarrel sponges	Total near flat sponges	Total near foliose sponges	Total near vase sponges	Total on transect	Total near shelf sponges	Total on transect	Total near gorgonians	Total on transect	Total near black corals	Total on transect
Unknown *Sebastomus*	19*	17*	21*	16*	6	18*	6	0	7	38*	13
Swordspine rockfish *Sebastes ensifer*	16*	14*	15*	24*	6	16*	6	92*	31	23*	13
Pygmy rockfish *Sebastes wilsoni*	9	12	4	8	16	7	14	0	7	4	16
Pinkrose rockfish *Sebastes simulator*	6	5	13*	2	1	8	1	0	1	3	1
Squarespot rockfish *Sebastes hopkinsi*	6	9	2	4	24	4	24	0	30	4	17
Shortspine combfish *Zaniolepis frenata*	5	4	6	5	2	6	2	4	1	8	5
Bank rockfish *Sebastes rufus*	4*	3	6*	13*	1	5*	1	0	2	4	2
Halfbanded rockfish *Sebastes semicinctus*	2	5	2	0	3	5	3	0	<1	4	4
Unknown rockfishes *Sebastes* spp	4	4	3	1	2	3	2	0	2	4	2
Juvenile rockfish *Sebastes* spp	3	3	1	3	18	1	22	0	10	1	12
Shortbelly rockfish *Sebastes jordani*	2	2	4*	1	1	3	1	0	1	1	2
Unidentified combfishes *Zaniolepis* spp	2	2	2	2	1	3	1	0	1	1	2
Cowcod *Sebastes levis*	2	2	2	9*	<1	2*	<1	0	<1	2*	<1
Starry rockfish *Sebastes constellatus*	2	2	1	2	1	2	1	0	1	0	1
Greenstriped rockfish *Sebastes elongatus*	2	1	2	3	1	2	1	0	1	0	1
Rosy rockfish *Sebastes rosaceus*	2	2	2	1	1	2	1	0	0	0	<1

continued

Table 4 (continued)

Taxa	Common sponges				Shelf sponges		Gorgonians		Black coral	
	Total near tarrel sponges	Total near flat sponges	Total near foliose sponges	Total near vase sponges	Total on transect	Total near shelf sponges	Total on transect	Total near gorgonians	Total on transect	Total near black corals	Total on transect
Dwarf-red rockfish *Sebastes rufinanus*	1	2	<1	2	3	1	3	0	<1	0	<1
Greenspotted rockfish *Sebastes chlorostictus*	1	1	<1	1	1	1	<1	0	<1	1	<1
Blackeye goby *Rhinogobiops nicholsii*	1	2	<1	0	4	<1	3	4	1	0	<1
Lingcod *Ophiodon elongatus*	2	1	1	0	<1	1	<1	0	<1	0	<1
Bocaccio *Sebastes paucispinis*	1	1	1	2	1	1	1	0	1	0	<1

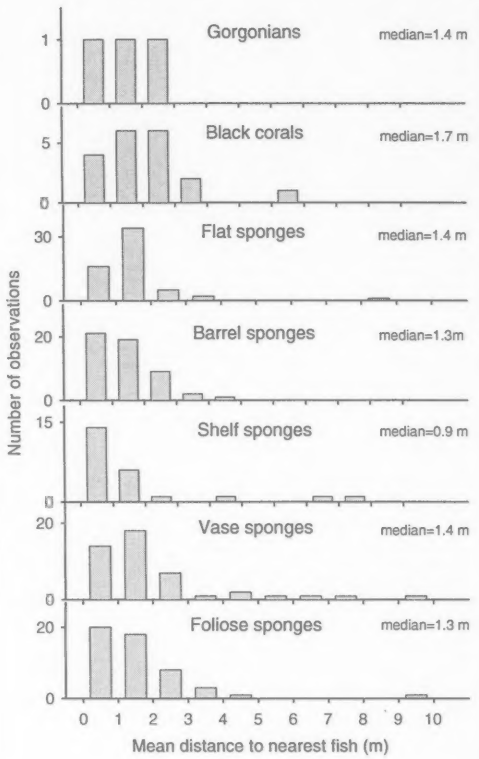

Figure 10
Mean nearest neighbor distances of fish species to gorgonians, black corals, and sponges.

relatively undisturbed megafaunal invertebrate communities in the southern California Borderlands, and supports the continued protection of these animals within the Cowcod Conservation Areas.

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Abstract—Larval and juvenile rockfishes (*Sebastes* spp.) are difficult to identify using morphological characters. We developed a key based on sizes of restriction endonuclease fragments of the NADH dehydrogenase-3 and -4 (ND3/ND4) and 12S and 16S ribosomal RNA (12S/16S) mitochondrial regions. The key makes use of variation in the ND3/ND4 region. Restriction endonuclease *Dde* I variation can corroborate identifications, as can 12S/16S variation. The key, based on 71 species, includes most North American taxa, several Asian species, and *Sebastolobus alascanus* and *Helicolenus hilgendorfi* that are closely related to rockfishes. Fifty-eight of 71 rockfish species in our database can be distinguished unequivocally, using one to five restriction enzymes; identities of the remaining species are narrowed to small groups: 1) *S. polyspinis*, *S. crameri*, and *S. ciliatus or variabilis* (the two species could not be distinguished and were considered as a single species) ; 2) *S. chlorostictus*, *S. eos*, and *S. rosenblatti*; 3) *S. entomelas* and *S. mystinus*; 4) *S. emphaeus*, *S. variegatus*, and *S. wilsoni*; and 5) *S. carnatus* and *S. chrysomelas*.

A key to selected rockfishes (*Sebastes* spp.) based on mitochondrial DNA restriction fragment analysis

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More than 100 species of rockfishes (genus *Sebastes*) are found worldwide (Kendall, 2000), and the majority are distributed along the Pacific coast of North America and in the northwest Pacific from the western Bering Sea south to Japan and Korea (Love et al., 2002). Sixty-five rockfish species are found along the California coast (Moser, 1996). Within the genus, there is a high degree of similarity of the morphological characters among many species. These similarities are in part due to recent divergence but also may have resulted from convergence of congeners occupying similar habitats.

Historically, species identification of *Sebastes* has been based on morphology; however, this approach is often insufficient, especially for identifying sympatric species, where there is considerable similarity in size and physical features and in pigmentation, as well as overlap in meristic characters. The difficulty in identifying *Sebastes* to species level exists for all developmental stages, but larvae are especially difficult to identify because they lack diagnostic characters (Kendall, 1991). Currently, only 15 species at the larval stage can be separated by physical characters such as body shape, pigmentation patterns, and head spine development (Love et al., 2002).

Rockfishes are important ecologically, and most species are economically valuable. *Sebastes* larvae form a large portion of ichthyoplankton collections and rank third or fourth in abundance among all fish larvae taken during California Cooperative Fisheries Investigations (CalCOFI) surveys that have covered the entire length of the California and Baja California coast and now cover waters off southern California. The ability to identify *Sebastes* accurately and efficiently at all developmental stages will greatly improve both our ability to learn about their life histories and our management and conservation ef-

forts. An increased understanding of life history variation can improve the systematic descriptions of *Sebastes* species, which has mostly been based on morphology.

Several methods have been developed to obtain species-specific information that supplements the use of morphological and meristic characters for species identification. Otolith microstructure and other hard structures have been used to distinguish the late-stage larvae and young-of-the-year pelagic juveniles of some *Sebastes* species (Laidig and Adams[1]; Laidig and Ralston, 1994; Laidig et al., 1996). Biochemical genetic methods have been used to identify adults (Barrett et al., 1966; Seeb, 1986), and may also be used to identify juveniles of some species (Seeb and Kendall, 1991). LeClair and Buckley (2001) used allozyme variation at 37 loci to positively identify 155 individuals of juvenile *Sebastes diploproa* and *Sebastes melanops*; but they were unable to identify another 29 individuals, partly because of a limited database. Despite some success with their use, allozymes often have low resolution, and there are complexes of species that cannot be distinguished by using allozymes alone (Seeb and Kendall, 1991).

DNA sequence-based methods have some advantages over the use of allozymes for the identification of juvenile fish species. They often have greater resolution, due in part to the fact that expression of some metabolic enzymes changes during development, whereas DNA sequences generally do not. DNA is less susceptible to degradation than the enzymes used in allozyme studies. Also, DNA sequence-based methods require a small amount of tissue sample, which is especially suited for work with samples from early life stages (e.g., Gray et al., 2006). Because it has a relatively high rate of substitution (Moritz et al., 1987), mitochondrial DNA (mtDNA) can be useful for distinguishing closely related species. Mitochondrial DNA is usually inherited maternally in vertebrates and does not recombine with paternal mtDNA if it is leaked into the zygote (Gyllensten et al., 1991; but see Rokas et al., 2003). Thus, the mtDNA sequence represents a matrilineal phylogeny and is often useful in delineating phylogenetic relationships of closely related species. Sequences of the mitochondrial cytochrome *b* gene were used to identify the pelagic young of *Sebastes constellatus* and *Sebastes ensifer* (Rocha-Olivares et al., 2000). Multiplex PCR of haplotype-specific regions of mtDNA has also been used to identify early stages of *Sebastes* species (Rocha-Olivares, 1998).

The objective of this study was to devise a key for species identification using mtDNA restriction fragment data from both the ND3/ND4 and 12S/16S regions. In a previous study, data for species-specific mtDNA restriction site variation in the ND3/ND4 region were presented for 15 *Sebastes* species (Gharrett et al., 2000). We have included data for 56 additional species using the ND3/ND4 and 12S/16S regions of the mtDNA as the target region of PCR-amplification. We have included specimens of *Helicolenus hilgendorfi* and *Sebastolobus alascanus* for contrast.

Materials and methods

Adult specimens of 71 species of rockfishes were collected from the Gulf of Alaska, the coastal waters of California and Baja California, and from the coast of Japan. Samples of *H. hilgendorfi* and *Sebastolobus alascanus*, species from two sister genera, were collected from Japanese coastal waters and the Gulf of Alaska, respectively, to provide outgroup comparisons. A sample of heart tissue from each specimen was preserved in either 95% ethanol or a solution that was 80% 0.25M ethylenediaminetetraacetic (EDTA) acid at pH 8 and saturated with NaCl (Seutin et al., 1991) and 20% dimethyl sulfoxide (DMSO). At least five individuals of each species were analyzed, except for a few species for which less than five specimens were available. We did not distinguish between *S. ciliatus* and *S. variabilis*, which had not yet been described (Orr and Blackburn, 2004), when we collected samples.

Total genomic DNA was isolated by using a Purgene DNA™ isolation kit (Gentra Systems, Inc., Minneapolis, MN). Two target regions of mtDNA were amplified by using the polymerase chain reaction. The ND3/ND4 region begins in the glycyl tRNA gene and spans the NADH-dehydrogenase subunit-3, arginyl tRNA, NADH-dehydrogenase subunit-4L, and NADH-dehydrogenase subunit-4 genes, ending in the histidyl tRNA gene. The 12S/16S region starts near the phenylalanyl tRNA end of the 12S rRNA gene, and runs through the valyl tRNA gene to near the leucyl tRNA end of the 16S rRNA gene. Primers for target regions have been used to amplify these regions in northern Pacific rockfish (Gharrett et al., 2001). The lengths for the amplified ND3/ND4 and 12S/16S regions are about 2385 and 2430 base pairs, respectively, based on the aggregate restriction maps.

Subsamples of the PCR products of each individual were digested with one or more of the restriction endonucleases *Bst*N I, *Bst*U I, *Cfo* I, *Dde* I, *Hin*d II, *Hin*f I, *Mbo* I, *Msp* I, *Rsa* I, and *Sty* I. Fragments were separated electrophoretically in 1.5% agarose gels (one part agarose [Sigma-Aldrich, St. Louis, MO] and two parts Synergel™ [Diversified Biotech Inc., Boston MA]) in 0.5×TBE buffer (TBE is 90mM Tris-boric acid, and 2 mm EDTA, pH 7.5). A 100 base-pair ladder provided molecular weight markers to estimate restriction fragment sizes. Gels were stained with ethidium bromide and photographed on an ultraviolet light transilluminator. Restriction fragments that could not be accurately measured from agarose gels were separated on 8% polyacrylamide gels and stained with SYBR Green I Nucleic Acid Stain™ (Molecular Probes, Eugene, OR) using a 25-bp ladder for a molecular weight standard.

[1] Laidig, T. E., and P. B. Adams (eds.). 1991. Methods used to identify pelagic juvenile rockfish (genus *Sebastes*) occurring along the coast of central California. NOAA-TM-NMFS-SWFSC-166, 180 p. NMFS Southwest Fisheries Science Center, 110 Shaffer Rd., Santa Cruz, CA 95060.

A restriction site map was created for each endonuclease by using all observed restriction fragment patterns and necessary double digests.

Results

Baseline data

Interspecific variation was observed for all enzymes in both mitochondrial regions, except for *Hind* II in the 12S/16S region. Intraspecific variation was observed for several enzymes. A total of 215 restriction sites were detected in the ND3/ND4 and 12S/16S regions (Appendix 1). Of the 215 sites, 97 were unique to *Sebastes* species, 21 were unique to *Sebastolobus alascanus*, seven were unique to *H. hilgendorfi*, and one was shared by *Sebastolobus alascanus* and *H. hilgendorfi*. The ND3/ND4 region had 141 sites, and the 12S/16S region had 74 sites. In the ND3/ND4 region, 36 sites were common in all haplotypes, whereas 46 occurred in only a single haplotype.

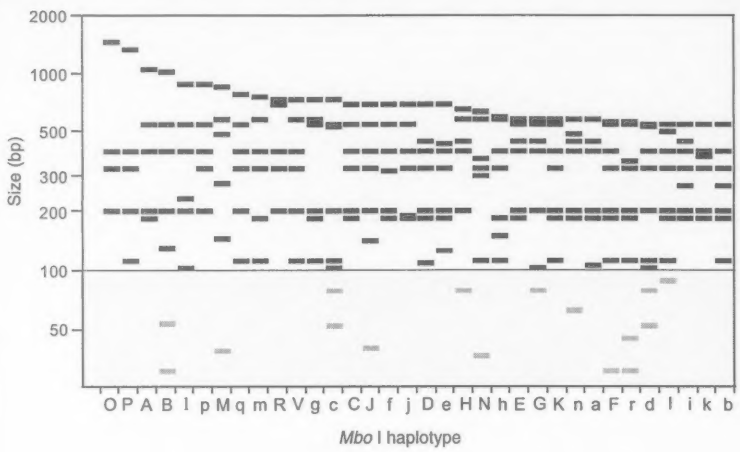

Figure 1

A mock gel showing expected fragment patterns for rockfish (*Sebastes* spp.) haplotypes as a result of digestion of the mitochondrial ND3/ND4 PCR product by restriction endonuclease *Mbo* I. The mobilities are the logarithm of the fragment sizes and separation was assumed to have taken place in 1.5% agarose gels (one part agarose and two parts Synergel™). The haplotypes correspond to the fragment sizes in Appendix 1 and to the haplotypes in Table 1 and the identification key. A haplotype may occur in more than one species. Most of the fragments that occurred in the shaded region would probably not be well resolved in an agarose gel.

A total of 132 composite haplotypes resulted from site differences in the two mtDNA regions (Table 1). The individuals of *Sebastes* species had 127 haplotypes; *Sebastolobus alascanus* had four haplotypes; and *H. hilgendorfi* had a single haplotype. Thirty-four of the 71 species displayed intraspecific variation and were represented by more than one composite haplotype; the remaining 37 species had a single composite haplotype.

Two pairs and three triplets of *Sebastes* species had identical composite haplotypes and could not be separated at the species level with this restriction site information (see identification key). These groups were 1) *S. carnatus* and *S. chrysomelas*; 2) *S. chlorostictus*, *S. eos*, and *S. rosenblatti*; 3) *S. ciliatus* or *variabilis*, *S. crameri*, and *S. polyspinis*; 4) *S. emphaeus*, *S. variegatus*, and *S. wilsoni*; and 5) *S. entomelas* and *S. mystinus*. Several pairs of haplotypes between variable *Sebastes* species were separated by a single restriction site. These included 1) *S. hopkinsi* and *S. ovalis*—*Dde* I; 2) *S. zacentrus* and the *S. emphaeus-S. variegatus-S. wilsoni* complex—*Rsa* I; and 3) *S. reedi* and the *S. ciliatus or variabilis-S. crameri-S. polyspinis* complex—*Mbo* I. The most variable species was *S. mystinus*, with five haplotypes. Five *Sebastes* species, *S. dalli*, *S. hubbsi*, *S. polyspinis*, *S. trivittatus*, and *S. zacentrus*, as well as *Sebastolobus alascanus*, had four haplotypes each. The remaining 58 *Sebastes* species have species-specific markers that allow unambiguous identification.

Use of the key

The key we developed for *Sebastes* species was based exclusively on variation in the ND3/ND4 region because it required only a single PCR amplification and variation in the 12S/16S region contributed no additional resolution between species to that provided by the ND3/ND4 region. The key is not a dichotomous key, but it is applied in the same way that taxonomic keys are applied. The first step is to digest PCR-amplified DNA from the ND3/ND4 region with restriction endonuclease *Mbo* I and to estimate the sizes of the fragments produced by separating the fragments on an agarose-Synergel™ gel. The best results will be achieved by using molecular weight markers, digitally photographing the gels, and estimating the fragment sizes with appropriate software. Alternatively, visual recognition of the fragment pattern can be accomplished by constructing a graphic key (e.g., Fig. 1 for *Mbo* I fragments) from fragment sizes predicted by the restriction site maps in Appendix 1. Note that in the figure, the logarithm of the size of the fragment (in base pairs) is used to estimate the mobility of a fragment (Sambrook et al., 1989). When the *Mbo* I haplotype has been identified, proceed to the next step. For example if the *Mbo* I digest results in haplotypes D or e, proceed to step g. in the key, which specifies digestion of another subsample of the ND3/ND4 PCR

Table 1

Composite haplotypes for *Sebastes* spp., *Helicolenus hilgendorfi*, and *Sebastolobus alascanus* in the ND3/ND4 and 12S/16S mtDNA regions. The haplotype codes refer to haplotypes in Appendix 1.

	ND3/ND4										12S/16S									
Species	*Bst*N I	*Bst*U I	*Cfo* I	*Dde* I	*Hin*d II	*Hin*f I	*Mbo* I	*Msp* I	*Rsa* I	*Sty* I	*Bst*N I	*Bst*U I	*Cfo* I	*Dde* I	*Hin*d II	*Hin*f I	*Mbo* I	*Msp* I	*Rsa* I	*Sty* I
aleutianus A	F	C	B	N	A	E	K	D	D	C	A	A	A	D	A	B	C	B	A	A
aleutianus B	F	C	B	N	A	F	K	D	D	C	A	A	A	D	A	B	C	B	A	A
alutus A	B	C	A	J	A	A	B	B	C	C	A	A	A	D	A	B	C	B	A	A
alutus B	B	C	A	J	A	A	B	B	C	C	A	A	A	D	A	A	C	B	A	A
atrovirens	O	B	D	b	C	G	j	C	B	I	A	A	A	D	A	B	A	A	A	A
auriculatus	F	B	D	v	A	G	k	C	B	A	A	A	A	D	A	B	A	A	A	A
aurora	B	C	D	m	A	A	b	L	A	e	A	A	A	D	A	A	C	B	A	A
babcocki A	F	C	D	G	A	A	G	B	B	C	A	A	A	C	A	B	C	B	B	A
babcocki B	F	C	D	F	A	A	H	B	B	C	A	A	A	C	A	B	C	B	B	A
borealis	F	C	D	D	A	A	F	B	B	C	B	A	A	A	A	B	C	A	C	A
brevispinis	G	C	D	C	A	A	K	D	B	D	A	A	A	D	A	A	C	A	C	A
capensis A	F	C	D	G	B	A	E	B	D	O	A	A	A	C	A	A	C	A	D	A
capensis B	F	C	D	F	B	A	E	B	D	O	A	A	A	C	A	A	A	A	D	A
capensis C	F	C	D	G	B	A	E	B	D	O	A	A	A	D	A	A	C	A	D	A
carnatus A	F	a	A	I	C	G	C	a	B	A	A	A	A	D	A	B	A	A	A	A
carnatus B	F	a	A	I	C	A	C	a	B	A	A	A	A	D	A	B	A	A	A	A
carnatus C	F	a	a	I	C	G	C	a	B	A	A	A	A	D	A	B	A	A	A	A
caurinus A	F	B	C	L	A	G	C	C	B	A	A	A	A	D	A	B	A	A	A	A
caurinus B	F	B	C	L	A	G	J	C	B	A	A	A	A	D	A	B	A	A	A	A
chlorosticus	F	B	D	G	B	A	E	B	C	C	A	A	A	C	A	B	C	A	D	A
chrysomelas A	F	a	A	I	C	G	C	a	B	A	A	A	A	D	A	B	A	A	A	A
chrysomelas B	F	a	A	I	C	G	C	a	B	A	A	A	A	D	A	B	A	A	C	A
ciliatus variabilis	A	D	A	M	A	A	F	B	F	C	A	A	A	D	A	B	C	A	A	A
constellatus A	F	C	D	G	F	A	E	E	D	M	G	A	A	C	A	A	C	E	D	A
constellatus B	F	C	D	G	F	A	E	E	D	M	A	A	A	C	A	A	C	A	D	A
crameri	A	D	A	M	A	A	F	B	F	C	A	A	A	D	A	B	C	A	A	A
dalli A	F	C	D	bb	b	G	p	C	B	g	A	A	A	D	A	H	A	A	A	A
dalli B	F	C	D	bb	b	G	l	C	B	g	A	A	A	D	A	H	A	A	A	A
dalli C	F	C	D	bb	b	G	p	C	a	b	A	A	A	D	A	H	A	A	A	A
dalli D	F	C	D	bb	b	G	p	C	B	g	A	A	A	D	A	B	A	A	A	A
diploproa	g	B	C	aa	A	A	C	M	C	C	A	A	A	C	A	B	G	F	A	A
elongatus A	W	C	I	h	A	A	C	M	P	C	A	A	A	D	A	B	I	A	A	A
elongatus B	W	C	I	h	A	A	A	M	P	C	A	A	A	D	A	B	I	A	A	A
emphaeus A	F	C	D	E	A	A	D	B	D	C	A	A	A	B	A	B	C	A	C	A
emphaeus B	F	C	D	E	A	A	D	B	B	C	A	A	A	B	A	B	C	A	C	A
ensifer A	F	C	D	F	B	Q	E	B	C	C	A	A	A	C	A	B	A	A	C	A
ensifer B	F	C	D	F	B	A	E	B	C	C	A	A	A	C	A	B	C	A	C	A
entomelas	F	C	J	l	A	A	E	a	B	C	A	A	A	D	A	A	A	F	B	A
eos	F	B	D	G	B	A	E	B	C	C	A	A	A	C	A	B	C	A	D	A
exsul	F	C	D	D	B	A	i	B	D	M	A	A	A	C	A	B	C	A	B	A
flavidus	E	C	D	H	A	C	A	B	A	C	A	A	A	A	A	B	C	B	D	A
gilli A	b	C	D	x	A	P	m	A	R	N	A	E	D	D	A	B	C	B	A	A
gilli B	b	C	D	x	A	P	K	A	R	N	A	E	D	D	A	B	C	B	A	A
goodei	Y	C	D	p	A	O	a	B	D	D	A	A	A	D	A	I	C	A	A	A
helvomaculatus A	F	C	D	K	B	A	A	B	C	C	A	A	A	C	A	B	B	A	D	A

continued

Table 1 (continued)

Species	ND3/ND4										12S/16S									
	BstN I	*BstU* I	*Cfo* I	*Dde* I	*Hind* II	*Hinf* I	*Mbo* I	*Msp* I	*Rsa* I	*Sty* I	*BstN* I	*BstU* I	*Cfo* I	*Dde* I	*Hind* II	*Hinf* I	*Mbo* I	*Msp* I	*Rsa* I	*Sty* I
helvomaculatus B	F	C	D	K	B	A	A	B	E	C	A	A	A	C	A	B	C	A	D	A
hopkinsi A	Z	C	A	n	A	g	K	B	C	e	A	A	A	C	A	B	C	A	B	A
hopkinsi B	Z	C	A	n	A	g	K	A	C	e	A	A	A	C	A	B	C	A	B	A
hopkinsi C	Z	C	A	u	A	g	g	B	C	e	A	A	A	C	A	A	C	A	B	A
hubbsi A	L	I	D	R	A	I	O	A	C	G	A	C	A	D	A	A	C	A	A	A
hubbsi B	M	I	D	S	A	I	O	A	C	G	A	C	A	D	A	A	C	A	A	A
hubbsi C	M	I	D	T	A	I	P	A	C	G	A	C	A	D	A	B	C	A	A	A
hubbsi D	M	I	D	S	A	F	O	A	C	G	A	C	A	D	A	A	C	A	A	A
inermis A	F	I	D	X	A	A	D	e	J	I	A	A	A	A	A	B	C	A	C	A
inermis B	F	H	D	X	A	A	D	e	J	I	A	A	A	A	A	B	C	A	C	A
inermis C	F	I	D	cc	A	I	D	e	J	I	A	A	A	A	A	B	C	A	C	A
jordani A	a	C	D	c	E	A	c	A	P	C	F	A	A	H	A	B	C	A	D	A
jordani B	a	C	D	c	E	A	d	A	P	C	F	A	A	H	A	B	C	A	D	A
jordani C	a	C	D	q	E	A	c	A	P	C	F	A	A	H	A	B	C	A	D	A
joyneri A	O	H	D	a	A	A	D	e	L	A	A	A	A	A	A	B	C	A	C	A
joyneri B	O	H	D	a	A	I	D	e	L	A	A	A	A	A	A	B	C	A	C	A
lentignosus	F	C	D	F	B	A	n	B	D	M	A	A	A	C	A	B	C	A	B	A
levis	B	A	g	d	A	A	q	d	B	C	A	A	A	D	A	B	C	A	D	A
macdonaldi	P	b	A	e	A	b	C	g	D	C	B	A	A	G	A	G	C	A	A	D
maliger	F	B	D	L	C	D	C	C	B	A	A	A	A	D	A	B	A	A	B	A
melanops A	D	C	D	F	A	B	E	A	A	C	A	A	A	D	A	B	C	B	D	A
melanops B	D	C	D	F	A	B	E	B	A	C	A	A	A	D	A	B	C	B	D	A
melanostomus A	O	C	D	g	A	A	K	A	O	C	A	A	A	D	A	B	C	B	C	A
melanostomus B	O	C	D	g	A	A	K	A	B	C	A	A	A	D	A	B	C	B	C	A
miniatus A	F	C	K	r	C	A	e	B	A	C	A	A	A	C	A	A	C	A	A	A
miniatus B	F	C	K	r	C	N	e	B	A	C	A	A	A	C	A	A	C	A	A	A
mystinus A	F	C	J	l	A	A	E	a	B	C	A	A	A	D	A	A	A	B	B	A
mystinus B	B	C	J	l	A	C	E	D	B	C	A	A	A	D	A	A	A	A	B	A
mystinus C	F	C	J	l	A	A	E	a	B	e	A	A	A	D	A	A	A	B	B	A
mystinus D	B	C	J	l	A	A	E	D	B	C	A	A	A	D	A	A	A	B	B	A
mystinus E	B	C	J	l	A	C	E	D	B	C	A	A	A	D	A	A	A	B	B	A
nebulosus A	F	G	A	Z	A	G	C	C	B	A	A	A	A	D	A	B	A	A	A	A
nebulosus B	F	G	A	Z	B	G	C	C	B	A	A	A	A	D	A	B	A	A	A	A
nigrocinctus	F	C	D	c	A	A	G	B	B	C	A	A	A	C	A	B	C	A	B	A
nivosus	N	A	D	U	A	I	C	B	I	A	A	A	A	G	A	A	C	A	A	C
ovalis	Z	C	A	o	A	g	K	B	C	e	A	A	A	C	A	B	C	A	B	A
paucispinis A	F	A	b	c	A	A	V	A	b	d	A	A	A	D	A	B	C	A	B	A
paucispinis B	F	A	b	c	g	A	V	A	b	d	A	A	A	D	A	B	C	A	B	A
phillipsi	B	C	D	m	A	A	b	B	B	e	A	A	A	D	A	B	C	B	A	A
pinniger	F	C	D	s	A	A	C	B	B	A	A	A	A	D	A	A	C	A	A	A
polyspinis A	A	D	A	M	A	A	F	B	F	C	A	A	A	D	A	B	C	A	A	A
polyspinis B	A	J	A	f	A	A	F	A	F	C	A	A	A	D	A	B	C	A	A	A
polyspinis C	A	D	A	M	A	A	F	B	F	C	A	A	A	I	A	B	C	A	A	A
polyspinis D	V	D	A	M	A	A	F	B	N	C	A	A	A	D	A	B	C	A	A	A
proriger	F	C	D	E	A	A	K	E	B	C	A	A	A	D	A	B	C	A	C	A
rastrelliger	e	C	C	v	A	a	A	C	B	A	A	A	A	D	A	B	A	A	A	A
reedi	A	D	A	M	A	A	r	B	F	C	A	A	A	D	A	B	C	A	A	A

continued

Table 1 (continued)

Species	ND3/ND4										12S/16S									
	*Bst*N I	*Bst*U I	*Cfo* I	*Dde* I	*Hind* II	*Hinf* I	*Mbo* I	*Msp* I	*Rsa* I	*Sty* I	*Bst*N I	*Bst*U I	*Cfo* I	*Dde* I	*Hind* II	*Hinf* I	*Mbo* I	*Msp* I	*Rsa* I	*Sty* I
rosaceus	F	C	D	k	B	A	E	B	B	C	A	A	A	C	A	B	C	A	B	A
rosenblatti	F	B	D	G	B	A	E	B	C	C	A	A	A	C	A	B	C	A	D	A
ruberrimus A	C	C	A	B	A	D	I	B	D	C	A	A	A	D	A	C	C	A	A	A
ruberrimus B	C	C	D	B	A	D	I	B	D	C	A	A	A	D	A	C	C	A	A	A
rubrivinctus	g	C	D	D	A	A	E	b	B	C	A	A	A	C	A	B	C	B	B	A
rufus A	F	C	D	aa	A	A	F	B	B	e	E	A	A	C	A	B	C	D	D	A
rufus B	F	C	D	aa	A	A	F	B	B	e	E	A	A	C	A	B	C	D	B	A
rufus C	F	C	D	aa	A	A	C	B	B	e	E	A	A	C	A	B	C	D	B	A
saxicola	O	C	D	t	A	G	f	B	C	C	G	F	C	D	A	B	C	A	A	A
semicinctus	X	C	D	j	A	C	p	A	Q	L	A	D	A	A	A	A	H	A	A	A
serrenoides A	D	C	D	D	B	C	f	B	P	C	A	A	A	D	A	B	C	B	D	A
serrenoides B	D	C	C	F	B	C	f	B	P	C	A	A	A	D	A	B	C	B	D	A
serriceps A	F	C	D	y	A	g	E	B	B	C	A	A	A	C	A	B	C	A	B	A
serriceps B	F	C	D	z	A	g	E	B	B	C	A	A	A	C	A	B	C	A	B	A
simulator	F	K	D	F	A	A	E	B	C	C	A	A	A	C	A	B	C	A	D	A
spinorbis	O	C	D	D	B	A	E	B	D	M	A	A	A	C	A	B	C	A	B	A
taczanowski A	P	A	D	W	A	I	R	H	C	I	A	A	A	A	A	D	C	B	A	C
taczanowski B	P	A	D	W	C	I	R	H	C	I	A	A	A	A	A	D	C	B	A	C
taczanowski C	P	A	D	W	C	I	R	H	C	I	A	A	A	A	A	D	C	A	A	C
thompsoni	O	H	D	Y	A	A	D	e	L	I	A	A	A	A	A	B	C	A	C	A
trivittatus A	F	A	D	V	A	F	K	E	C	H	A	A	A	A	A	E	B	B	A	C
trivittatus B	F	A	D	V	A	D	K	E	C	H	E	A	A	A	A	E	B	C	A	C
trivittatus C	F	A	D	V	B	D	K	E	B	H	E	A	A	A	A	E	B	C	A	C
trivittatus D	F	A	D	V	B	D	K	E	C	H	E	A	A	A	A	E	B	C	A	C
umbrosus	F	C	D	w	B	A	n	B	D	M	A	A	A	C	A	B	A	A	B	A
variegatus	F	C	D	E	A	A	D	B	B	C	A	A	A	B	A	B	C	A	C	A
vulpes A	B	D	D	V	A	A	h	B	C	G	A	C	A	A	A	E	B	B	A	B
vulpes B	B	D	D	V	A	A	h	B	C	H	A	C	A	A	A	E	B	B	A	B
vulpes C	B	D	D	i	A	A	h	B	C	H	A	C	A	A	A	E	B	B	A	B
wilsoni A	F	C	D	E	A	A	D	B	B	C	A	A	A	B	A	B	C	A	C	A
wilsoni B	F	C	A	i	A	A	D	B	B	C	A	A	A	B	A	B	C	A	C	A
zacentrus A	F	A	D	E	A	A	D	B	C	C	A	A	A	B	A	B	C	A	C	A
zacentrus B	F	A	D	A	A	A	D	B	C	C	A	A	A	B	A	B	C	A	C	A
zacentrus C	F	C	D	E	A	A	D	B	C	C	A	A	A	B	A	B	C	A	C	A
zacentrus D	F	A	D	E	A	A	D	B	C	B	A	A	A	B	A	B	C	A	C	A
Helicolenus hilgendorfi	J	G	F	Q	A	A	N	G	H	A	D	B	B	F	B	B	F	B	A	A
Sebastolobus alascanus A	H	E	E	O	A	H	M	F	G	F	C	B	B	E	A	D	E	B	E	B
Sebastolobus alascanus B	I	E	E	O	A	H	M	F	G	F	C	B	B	E	A	D	E	B	E	B
Sebastolobus alascanus C	H	F	E	O	A	H	M	F	G	E	C	B	B	E	A	D	E	B	E	B
Sebastolobus alascanus D	H	E	E	P	A	H	M	F	G	F	C	B	B	E	A	D	E	B	E	B

Identification key

Key for distinguishing *Sebastes* spp. using ND3/ND4 restriction site information. This is one of many possible schemes to identify unknown specimens to species level or to small groups of species. Because new intraspecific variation may be encountered, it is wise to confirm the identification by cutting the DNA using an additional one or two enzymes.

1. Digest mtDNA with *Mbo* I.
2. Place resulting restriction fragment patterns (haplotypes) in one of the following categories (see Appendices 1 and 2 for details).
   - a. O—*S. hubbsi* A, B, D.
   - b. P—*S. hubbsi* C.
   - c. A or B.
     Digest mtDNA with *Bst*N I.
     - i. B—*S. alutus*
     - ii. W—*S. elongatus* A
     - iii. E—*S. flavidus*
     - iv. F—*S. helvomaculatus*
     - v. C—*S. rastrelliger*
   - d. p or l.
     Digest mtDNA with *Bst*N I.
     - i. X—*S. semicinctus*
     - ii. F—*S. dalli*
   - e. R—*S. taczanowski*
   - f. q, V, C, J, f, j.
     Digest with *Bst*N I.
     - i. B—*S. levis*
     - ii. N—*S. nivosus*
     - iii. O.
       Digest mtDNA with *Bst*U I.
       1. B—*S. atrovirens*
       2. C—*S. saxicola*
     - iv. W—*S. elongatus*
     - v. g—*S. diploproa*
     - vi. F.
       Digest mtDNA with *Bst*U I.
       1. A—*S. paucispinis*
       2. C.
          Digest mtDNA with *Dde* I.
          - *a.* s—*S. pinniger*
          - *b.* aa—*S. rufus* C
       3. a—*S. carnatus*, *S. chrysomelas*
       4. B.
          Digest mtDNA with *Cfo* I.
          - *a.* D—*S. maliger*
          - *b.* C—*S. caurinus*
       5. G—*S. nebulosus*
     - vii. P—*S. macdonaldi*
   - g. D or e.
     Digest mtDNA with *Bst*N I.
     - i. F.
       Digest mtDNA with *Dde* I.
       1. A—*S. zacentrus* B
       2. E.
          Digest mtDNA with *Rsa* I.
          - *a.* C—*S. zacentrus* A,C,D
          - *b.* B—*S. emphaeus* B, *S. variegatus*, *S. wilsoni* A (identical)
          - *c.* D—*S. emphaeus* A
       3. cc—*S. inermis* C
       4. X or r.
          Digest mtDNA with *Bst*U I.
          - *a.* C—*S. miniatus*
          - *b.* H—*S. inermis* A
          - *c.* I—*S. inermis* B
       5. i—*S. wilsoni* B
     - ii. O.
       Digest mtDNA with *Dde* I.
       1. Y—*S. thompsoni*
       2. a—*S. joyneri*
   - h. c—*S. jordani* A
   - i. H—*S. babcocki* B
   - j. g—*S. hopkinsi* A
   - k. h—*S. vulpes*
   - l. E.
     Digest mtDNA with *Bst*N I.
     - i. B—*S. mystinus* A
     - ii. O—*S. spinorbis*
     - iii. D—*S. melanops*
     - iv. F or g.
       Digest mtDNA with *Dde* I.
       1. l.
          Digest mtDNA with *Bst*N I.
          - *a.* F—*S. entomelas*/ *S. mystinus* A, C
          - *b.* B—*S. mystinus* B, D
       2. D—*S. rubrivinctus*
       3. G.
          Digest mtDNA with *Bst*U I.
          - *a.* C—*S. constellatus*
          - *b.* B—*S. chlorostictus*, *S. eos*, *S. rosenblatti* (identical)
       4. z—*S. serriceps* A
       5. k or y.
          Digest mtDNA with *Hin*d II.
          - *a.* A—*S. serriceps* B
          - *b.* B—*S. rosaceus*
   - m. G—*S. babcocki* A
   - n. K.
     Digest mtDNA with *Bst*N I.
     - i. Z—*S. hopkinsi* B
     - ii. O—*S. melanostomus*
     - iii. F.
       Digest mtDNA with *Hinf* I.
       1. A—*S. proriger*
       2. D—*S. trivittatus*
       3. E—*S. aleutianus* A
       4. B or F.
          Digest mtDNA with *Bst*U I.
          - *a.* A—*S. trivittatus*
          - *b.* C—*S. aleutianus* B
     - iv. G—*S. brevispinis*
   - o. a—*S. goodei*
   - p. F.
     Digest mtDNA with *Bst*N I.
     - i. A—*S. crameri*, *S. ciliatus*, *S. polyspinis* A, B (first 2 spp. and *S. polyspinis* A identical)
       Digest mtDNA with *Bst*U I.
       1. D—*S. crameri*, *S. ciliatus*, *S. polyspinis* A
       2. J—*S. polyspinis* B
     - ii. F.
       Digest mtDNA with *Dde* I.
       1. D—*S. borealis*
       2. aa—*S. rufus* A, B
     - iii. V—*S. polyspinis* C
   - q. d—*S. jordani* B
   - r. I—*S. ruberrimus*
   - s. i—*S. exsul*
   - t. b—*S. aurora*
   - u. k—*S. auriculatus*
   - v. r—*S. reedi*

product with endonuclease *Bst*N I. Continue digesting with the specified restriction endonucleases, identifying the resultant haplotype, and continue to the next step until the species has been identified. Between one and five restriction enzymes will be needed to achieve the separation. Examining the fragment patterns of additional restriction enzymes can increase confidence in the identifications (see key).

Although variation observed in the 12S/16S region was not used in the key, it does provide alternatives to species identification that may be useful for resolving the identification of some species or for corroborating identifications, especially when there is intraspecific variation that has not been previously observed for the enzymes applied in resolving species. In particular, *Rsa* I, *Dde* I, and *Mbo* I exhibited substantial interspecific variation in the 12S/16S region. In addition, if one has reduced the possible species by other means, a single 12S/16S digest can be used in some instances. For example, *S. aleutianus* and *S. borealis* differ in fragments produced by *Bst*N I and by *Rsa* I, and *S. caurinus* and *S. maliger* also differ in fragments produced by *Rsa* I.

Discussion

Restriction site analysis of mtDNA is a simple and effective tool for identifying juveniles of *Sebastes* species. Because inexpensive equipment is used to obtain data from restriction fragment patterns, the analyses can be conducted in most laboratories, including many high school laboratories.

Although the restriction fragment key identified most (58 or 81.7%) of the 71 different rockfish species we evaluated, it failed to identify 13 species. The identities of those 13, however, were narrowed to five small groups of species. One group, *S. carnatus* and *S. chrysomelas*, are very closely related; they differ obviously only in body coloration as adults: *S. carnatus* has flesh-colored blotches on an olive-brown background, and *S. chrysomelas* has yellow blotches on a black background (Love et al., 2002). They are included in the subgenus *Pteropodus* (Kendall, 2000). Another group includes *S. chlorostictus*, *S. eos*, and *S. rosenblatti*, which are also morphologically similar, occur sympatrically, and are closely related (Chen, 1971; Love, 1996; Rocha-Olivares et al., 1999; Love et al., 2002); all are members of the subgenus *Sebastomus*. Estimates of divergence times of the subgenus *Sebastomus* suggest that the three species are the result of the most recent speciation events within the subgenus, which may have begun less than 140,000 years ago (Rocha-Olivares et al., 1999). Members of a third group, *S. emphaeus-S. variegatus-S. wilsoni*, are assigned to the subgenus *Allosebastes* (Kendall, 2000). The relationships between species in the other two unresolved groups are not as clear because the subgenera of the members differ. In one of those groups, *S. entomelas* is in subgenus *Acutomentum* and *S. mystinus* is in subgenus *Sebastosomus*. In the other subgroup, *S. polyspinis* remains unassigned to a subgenus, *S. crameri* is in *Eosebastes*, and *S. ciliatus* (subgenus *Sebastosomus*) has only recently been separated from *S. variabilis* (Orr and Blackburn, 2004). The similarities observed may actually reflect genetic relationships because the subgenera assignments are probably inaccurate reflections of phylogeny (Kendall, 2000). Our inability to resolve within those five groups of rockfish to species indicates the need for additional markers. Approaches for obtaining such markers include screening additional regions of the mtDNA and application of additional restriction enzymes. If additional mtDNA regions and restriction enzymes do not provide species-specific information, other molecular techniques such as microsatellites should be considered.

We applied the baseline data from which our key was developed to identification of recently extruded rockfish larvae in Southeast Alaskan waters (Gray et al., 2006). That application, which was made while the key presented in this article was being developed, evolved over subsequent years of application. The key was also used to delineate juvenile rockfishes from the southern California Bight (Li et al., in press). We were able to identify all the specimens to species or to narrow identification to one of the five small groups of genetically similar species. The identifications of juvenile rockfish were concordant with genetic and morphological criteria, except that the genetic key did resolve species in the closely related subgenus *Sebastomus*, which could not be resolved with morphological criteria. The larval specimens, in contrast, could not be identified to species with morphological criteria. We detected previously unobserved intraspecific variation in both studies as well. Neither the variation observed in the larval and juvenile studies, the intraspecific variation observed in developing the key, nor intraspecific variation observed among large numbers of individuals of several species examined as part of a population genetic analysis (Li, 2004) obscured species detection.

Molecular genetic keys can remove much of the guesswork for species determination in ichthyoplankton and juvenile surveys. Because DNA-based characters remain constant throughout the life of an individual, genetic divergence can provide unequivocal markers for species delineation. For specific questions involving the identification of small numbers of species, such as distinguishing between the two sibling species included in *S. aleutianus* (Gharrett et al., 2005), it is possible to develop assay methods (e.g., single nucleotide polymorphism—referred to as SNP (Collins et al., 1996), that can rapidly identify large numbers of specimens. Finally, such a key can be used to identify larval and juvenile species of *Sebastes* so that the variation in the morphological characters can be determined.

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Appendix 1

Restriction site locations for *Sebastes* spp., *Sebastolobus alascanus*, and *Helicolenus hilgendorfi* in the ND3/ND4 and 12S/16S mtDNA regions. Letters denote haplotypes (restriction digest patterns). "X" denotes presence of site. "O" denotes absence of site.

ND3/ND4 *Bst*N I sites	haplotypes																							
	A	B	C	D	E	F	G	H	I	J	L	M	N	O	P	V	W	X	Y	Z	a	b	c	g
112	O	X	X	X	X	X	X	X	X	X	O	X	X	X	X	O	O	X	X	O	X	X	X	X
291	O	O	O	O	X	O	O	O	O	O	O	O	X	O	O	O	O	O	O	O	O	X	X	O
551	O	O	O	O	O	O	O	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
829	X	X	O	X	X	X	X	X	X	X	X	X	X	X	X	X	X	O	O	O	O	X	X	X
1035	O	O	O	O	O	O	O	O	O	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O
1214	X	X	X	O	O	X	X	O	O	O	O	O	X	O	X	O	X	O	X	O	X	X	X	X
1261	O	O	O	O	O	O	O	O	X	O	X	X	X	O	O	O	O	O	O	O	O	O	O	O
1495	O	O	O	O	O	O	O	O	O	O	X	X	O	O	O	O	O	O	X	O	O	O	O	O
1694	O	O	X	X	X	X	X	O	O	O	O	O	O	X	X	O	X	X	O	X	O	O	X	X
1982	O	O	O	X	X	O	X	O	O	O	O	O	O	O	O	O	O	O	X	O	O	O	O	O
2010	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
2121	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	O	O	O	O	O	O	X	O	O
2255	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
2325	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	O	X	O

ND3/ND4 *Bst*U I sites	haplotypes												
	A	B	C	D	E	F	G	H	I	J	K	a	b
4[1]	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
344	O	O	O	O	O	X	O	O	O	O	O	O	O
668	O	O	O	O	O	O	O	X	O	O	O	O	O
710	O	O	O	O	O	O	O	O	X	O	O	O	X
1499	O	O	O	O	X	O	O	O	O	O	O	O	O
1854	O	O	O	X	O	O	O	O	O	X	X	O	O
2025	O	X	X	O	O	O	O	X	O	O	X	X	X
2109	O	O	O	O	O	O	O	O	X	X	O	X	O
2306	O	X	O	O	X	X	X	O	O	O	O	X	O

[1] The site at 4 is in the primer region.

Cfo I sites	haplotypes											
	A	B	C	D	E	F	I	J	K	a	b	g
709	X	O	X	X	X	X	X	X	X	X	X	X
1221	O	O	O	O	X	X	X	O	O	O	O	O
1436	O	O	O	O	X	O	O	O	O	O	O	O
1464	O	O	O	O	O	O	O	X	O	X	O	O
1472	O	O	O	O	O	O	O	O	O	O	X	X
1741	O	X	X	X	O	O	X	X	X	O	X	X
1762	O	O	O	O	O	O	O	O	X	O	O	O
1813	O	O	X	O	O	O	O	O	O	O	X	O

ND3/ND4 *Dde* I sites	haplotypes																							
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	
65	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	O	O	O	O	O	O	
195	X	O	O	X	X	X	X	X	X	X	X	X	X	X	O	O	O	O	O	O	X	X	O	
266	O	O	O	O	O	O	X	O	X	O	O	X	O	O	X	X	X	O	O	O	O	O	O	
298	X	X	O	X	X	X	X	O	X	X	X	X	X	X	O	O	X	O	O	O	O	X	X	
336	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
374	X	X	X	O	X	O	O	O	O	X	O	O	X	O	O	O	O	X	X	X	O	X	O	

continued

Appendix 1 (continued)

ND3/ND4 (continued) *Dde* I sites	haplotypes A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
590	O	X	X	X	X	X	X	X	X	X	X	X	X	X	O	O	X	X	X	X	X	X	X
624	O	O	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
663	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	O	O	O	O	O	O
695	O	O	O	O	O	O	O	O	X	O	O	X	O	O	O	O	O	O	O	O	O	O	O
754	O	O	O	O	O	O	O	O	X	O	O	X	X	O	O	O	X	O	O	O	X	X	X
1144	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
1190	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	X	X	O	O	O
1257	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	O	O	O	O	O	O
1409	O	O	O	O	O	O	O	O	O	O	X	O	O	O	O	O	O	O	O	O	O	O	O
1560	O	O	O	O	O	O	O	O	X	O	O	X	O	O	O	O	O	X	O	O	O	O	O
1577	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	X	X	O	O	O	O	O	O
1587	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
1601	X	O	X	X	X	X	X	X	X	X	X	X	X	X	O	O	O	X	X	X	X	X	X
1696	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	O	O	O	O	O	O
1722	O	O	O	O	O	O	O	O	O	X	O	O	O	X	X	X	X	O	O	O	O	O	O
1779	O	O	X	O	O	O	O	O	X	O	O	X	X	O	O	O	O	X	X	X	O	O	X
1795	O	O	O	O	O	X	X	X	X	X	X	X	X	O	O	O	X	O	O	O	X	X	X
1912	X	X	X	X	X	X	X	X	O	X	X	X	X	X	O	O	X	X	X	O	X	X	X
2091	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	O	O	O	O	O	X	O	O
2122	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
2188	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	X	O	O	O	O	O	O	O
2374[2]	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)

ND3/ND4 *Dde* I sites	haplotypes (continued) X	Y	Z	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t
65	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
195	O	O	X	X	O	X	X	O	X	X	X	X	X	X	X	X	X	X	O	X	X	X	X
266	O	O	X	O	X	O	O	O	O	O	O	O	X	O	O	O	O	O	X	O	O	O	X
298	X	O	X	O	X	X	X	X	X	X	O	X	X	X	O	X	X	X	X	X	O	X	O
336	X	O	X	O	X	X	X	X	X	X	X	X	X	X	O	X	O	O	X	X	X	X	X
374	X	X	O	X	O	O	O	X	X	X	O	X	O	O	O	O	O	O	O	O	X	X	O
590	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
624	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
663	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
695	O	O	X	O	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
754	X	O	X	O	O	X	O	O	X	O	O	X	O	X	O	X	X	X	O	X	O	O	O
1144	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	O	O	O
1190	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
1257	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
1409	O	O	O	O	O	O	O	O	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O
1560	X	X	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
1577	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
1587	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	O
1601	X	X	X	X	X	O	X	O	X	X	X	X	O	X	X	X	X	X	O	O	X	X	X
1696	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
1722	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
1779	X	X	X	X	X	O	O	O	X	O	X	O	X	O	O	O	O	O	O	O	O	O	X
1795	X	X	X	X	X	X	X	X	X	X	X	O	X	O	X	X	O	X	X	X	X	X	X
1912	X	X	X	X	O	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2091	O	O	O	O	O	O	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
2122	O	O	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
2188	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
2374[2]	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)

continued

Appendix 1 (continued)

ND3/ND4 *Dde* I sites	haplotypes (continued)									Hind II sites	haplotypes						
	u	v	w	x	y	z	aa	bb	cc		A	B	C	E	F	b	g
65	O	O	O	O	O	O	O	O	O	308	O	O	O	O	O	O	X
195	X	X	X	O	X	X	X	X	X	320	O	O	O	O	X	O	O
266	O	X	O	O	O	O	O	X	O	324	O	O	O	O	O	X	O
298	X	X	X	O	X	X	X	X	O	978	O	O	O	O	O	X	O
336	O	X	X	X	X	X	X	X	O	1007	O	X	O	O	X	O	O
374	O	O	O	O	O	O	O	O	X	1071	O	O	O	X	O	O	O
590	O	X	X	X	X	X	X	X	X	1091	O	O	X	O	O	O	O
624	O	O	O	X	O	O	O	O	O								
663	O	O	O	O	O	O	O	O	O								
695	O	X	O	O	O	O	O	X	O								
754	X	O	O	O	O	O	X	O	O								
1144	O	O	O	O	O	O	O	O	O								
1190	O	O	O	O	O	O	O	O	O								
1257	O	O	O	O	O	O	O	O	O								
1409	O	O	O	O	X	X	O	O	O								
1560	O	X	O	O	O	O	O	O	O								
1577	O	O	O	O	O	O	O	O	O								
1587	O	O	O	O	O	O	O	O	O								
1601	X	X	O	X	X	O	X	X	X								
1696	O	O	O	O	O	O	O	O	O								
1722	O	O	O	O	O	O	O	O	O								
1779	O	X	O	O	O	O	X	X	X								
1795	X	X	X	X	O	O	X	X	X								
1912	X	X	X	X	X	X	X	X	X								
2091	O	O	O	O	O	O	O	O	O								
2122	O	O	O	O	O	O	O	O	O								
2188	O	O	O	O	O	O	O	O	O								
2374[2]	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)								

[2] The site at 2374 is in the primer region.

ND3/ND4 *Hinf* I sites	haplotypes															
	A	B	C	D	E	F	G	H	I	N	O	P	Q	a	b	g
43	O	O	O	O	O	O	O	O	O	O	O	X	O	O	O	O
130	O	X	O	O	O	X	O	O	X	O	X	X	O	O	O	O
183	O	O	O	O	O	O	O	O	O	X	O	O	O	O	O	O
389	O	X	X	O	O	O	O	X	O	O	O	O	O	O	O	O
472	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X
494	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
621	O	O	O	O	O	O	O	O	O	O	O	O	O	X	O	O
853	O	O	O	O	O	O	O	X	O	O	O	O	O	O	O	O
999	O	O	O	O	O	O	O	O	O	O	O	X	O	O	O	O
1017	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	O
1342	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	O
1448	O	O	O	O	O	O	O	X	O	O	O	O	O	O	O	O
1537	O	O	O	O	O	O	X	O	O	O	X	O	O	X	O	O
1755	O	O	O	O	X	X	O	O	O	O	O	O	O	O	O	O
1888	O	O	O	X	O	O	O	X	O	O	O	O	O	O	O	O
2011	O	O	O	O	O	O	O	O	O	O	O	O	X	O	O	O
2232	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

continued

Appendix 1 (continued)

ND3/ND4 *Mbo* I sites	haplotypes																					
	A	B	C	D	E	F	G	H	I	J	K	M	N	O	P	R	V	a	b	c	d	e
150	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
190	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
198	X	X	X	X	X	X	X	X	X	X	X	O	O	X	X	X	X	X	X	X	X	X
261	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
272	O	O	O	O	O	O	O	O	O	O	O	X	O	O	O	O	O	O	O	O	O	O
302	O	O	O	O	O	O	O	O	O	O	O	O	X	O	O	O	O	X	O	O	O	O
632	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X
648	O	O	O	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
758	X	X	X	X	X	X	X	O	X	X	X	X	O	O	O	O	O	X	X	X	X	X
797	O	O	O	O	O	O	O	O	O	O	O	X	O	O	O	O	O	O	O	O	O	O
861	O	O	O	O	O	O	X	X	O	O	O	O	O	O	O	O	O	O	O	X	X	O
886	O	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
900	O	O	O	O	O	O	O	O	O	X	O	O	O	O	O	O	O	O	O	O	O	O
940	X	X	X	X	X	X	X	X	X	X	X	X	X	O	O	X	X	X	X	X	X	X
971	O	X	O	O	O	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
1029	O	O	O	O	O	O	O	O	X	O	O	O	O	O	O	O	O	O	O	O	O	O
1270	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	O	O	O
1481	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	X	O
1534	O	O	O	O	X	X	X	X	X	O	X	X	X	O	X	O	X	X	X	X	X	O
1647	O	O	X	X	O	X	O	O	X	X	X	O	X	X	X	X	X	O	X	X	X	X
1658	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
1748	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
1979	X	X	X	X	X	X	X	X	X	X	X	O	X	X	X	X	X	X	X	O	X	X
2016	O	O	O	O	O	O	O	O	O	O	O	O	X	O	O	O	O	O	O	O	O	O
2339	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O

ND3/ND4 *Mbo* I sites	haplotypes (continued)											
	f	g	h	i	j	k	l	m	n	p	q	r
150	O	O	X	O	O	O	O	O	O	O	O	O
190	O	O	O	O	X	O	O	O	O	O	O	O
198	X	X	O	X	X	X	X	O	X	X	X	X
261	O	O	O	O	O	O	O	O	X	O	O	O
272	O	O	O	O	O	O	O	O	O	O	O	O
302	O	O	O	O	O	O	O	O	O	O	O	O
632	O	O	O	O	O	O	O	O	O	O	O	O
648	O	O	O	O	O	O	O	O	O	O	O	O
758	X	X	X	X	X	X	X	X	X	X	X	X
797	O	O	O	O	O	O	O	O	O	O	O	O
861	O	O	O	O	O	O	O	O	O	O	O	O
886	O	O	O	O	O	O	O	O	O	O	O	O
900	O	O	O	O	O	O	O	O	O	O	O	O
940	X	X	X	X	X	X	O	X	X	O	O	X
971	O	O	O	O	O	O	O	O	O	O	O	X
1029	O	O	O	O	O	O	O	O	O	O	O	O
1270	O	O	O	X	O	X	O	O	O	O	O	O
1481	O	O	O	O	O	O	O	O	O	O	O	O
1534	O	X	X	X	O	O	O	X	X	O	X	X
1647	X	X	X	O	X	X	X	X	O	X	X	X
1658	X	O	O	O	O	O	O	O	O	O	O	O
1748	O	O	O	O	O	O	X	O	O	O	O	O
1979	X	O	X	X	X	X	X	X	X	X	X	X
2016	O	O	O	O	O	O	O	O	O	O	O	O
2339	O	O	O	O	O	O	O	O	O	O	O	X

continued

Appendix 1 (continued)

ND3/ND4 *Msp* I sites	haplotypes														
	A	B	C	D	E	F	G	H	L	M	a	b	d	e	g
30	X	X	X	X	X	X	X	X	X	O	X	X	X	X	X
933	O	O	O	O	O	X	O	O	O	O	O	O	O	O	O
1199	O	O	O	O	O	X	O	O	O	O	O	O	O	O	O
1230	O	O	O	O	O	O	O	O	O	O	O	O	X	O	O
1261	O	O	O	O	O	O	X	O	O	O	O	O	O	O	O
1541	O	O	O	O	O	O	O	X	O	O	O	X	O	O	X
1624	O	O	O	O	O	O	O	O	X	O	O	O	O	X	O
1738	X	X	X	X	X	X	X	O	X	X	X	X	X	X	X
1826	X	X	O	X	X	O	O	X	X	X	O	X	X	X	X
1844	O	O	O	X	X	O	O	O	O	O	O	O	X	O	O
2073	O	O	O	O	O	X	O	O	O	O	O	O	O	O	O
2110	O	X	X	O	X	O	O	O	O	O	O	O	X	X	X

Rsa I sites	haplotypes																
	A	B	C	D	E	F	G	H	I	J	L	N	O	P	Q	R	a	b
346	O	O	O	X	X	O	X	X	O	O	X	X	O	X	O	O	O	O
561	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	O	O
742	O	O	O	O	O	O	O	X	X	O	O	O	O	O	O	O	O	O
1077	O	O	O	O	O	X	O	O	O	O	X	X	O	O	O	O	O	O
1231	O	O	O	O	O	O	X	O	O	O	O	O	O	O	O	O	O	O
1321	O	O	O	O	O	O	O	O	O	O	O	O	X	O	O	O	O	O
1339	O	O	O	O	O	O	O	O	O	X	O	O	O	O	O	O	O	O
1357	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	O
1433	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	O
1492	O	O	O	O	O	O	O	X	O	O	O	O	O	O	O	O	O	O
1863	O	O	X	O	X	O	O	X	X	X	X	O	O	O	O	O	O	X
1985	O	X	X	X	X	X	X	X	X	X	X	X	X	O	X	X	X	O
2063	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	O	O	O

ND3/ND4 *Sty* I sites	haplotypes															
	A	B	C	D	E	F	G	H	I	L	M	N	O	b	d	e	g
49	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
108	O	O	O	O	O	O	O	O	O	O	X	O	O	X	O	O	X
194	O	O	O	X	X	X	O	O	X	O	O	X	O	X	O	O	O
365	X	X	X	X	O	O	X	X	X	X	X	X	O	X	O	X	X
391	O	O	O	O	X	X	O	O	O	X	O	O	O	O	O	O	O
534	X	O	X	X	X	X	O	O	X	X	X	X	X	X	O	X	X
1258	O	O	O	O	O	X	O	O	O	O	O	O	O	O	O	O	O
1570	O	X	X	X	X	X	O	O	O	X	X	O	X	O	X	X	O
1725	O	O	O	O	O	O	X	O	O	O	O	X	O	O	O	X	O
2311	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

12S/16S *Bst*N I sites	haplotypes						
	A	B	C	D	E	F	G
326	X	X	X	X	O	X	X
988	O	O	O	X	O	X	O
1105	O	O	O	O	O	X	O
1530	O	O	O	O	O	O	X
1687	O	O	X	O	O	O	O
1741	O	X	O	O	O	O	O
2416	X	X	X	X	X	X	X

Appendix 1 (continued)

12S/16S *Bst*U I sites	haplotypes A	B	C	D	E	F
87	X	X	X	X	X	X
600	X	O	X	X	O	X
664	X	X	X	X	X	X
729	X	X	O	O	X	X
1633	O	O	O	O	X	O
1872	X	X	X	X	X	X
2128	X	X	X	X	X	X
2191	O	O	O	X	O	X

Cfo I sites	haplotypes A	B	C	D
537	X	X	O	X
602	X	O	X	X
665	X	X	X	X
1630	O	O	O	X
1898	X	X	X	X
2268	X	X	X	X

Dde I sites	haplotypes A	B	C	D	E	F	G	H	I
44	X	X	X	X	X	X	X	X	X
55	X	X	X	X	X	X	X	X	X
976	X	X	X	X	X	O	O	O	X
1043	X	X	O	X	X	O	O	O	X
1056	X	X	O	X	O	O	X	O	X
1304	X	X	X	X	X	O	X	X	X
1339	O	O	O	O	O	O	O	O	X
1735	O	X	X	X	X	O	X	X	X
2181	X	O	X	X	X	O	O	X	X
2393	X	X	X	X	X	X	X	X	X

Hinf I sites	haplotypes A	B	C	D	E	G	H	I
982	O	O	X	O	O	O	O	X
1181	O	O	O	O	O	X	O	O
1291	X	X	X	O	O	X	X	X
1700	O	O	O	O	O	O	X	X
2094	O	X	X	O	X	O	X	X

Hind II sites	haplotypes A	B
1717	X	O

12S/16S *Mbo* I sites	haplotypes A	B	C	E	F	G	H	I
201	O	X	X	O	O	X	X	X
849	X	X	X	O	X	X	X	X
1015	X	X	X	X	O	O	X	X
1210	O	O	O	O	O	O	O	X
1403	X	X	X	X	X	X	X	X
1507	X	O	X	X	X	X	X	X
1637	O	O	O	O	O	O	X	O
1984	X	X	X	X	X	X	X	X
2059	X	X	X	X	X	X	X	X
2228	X	X	X	X	X	X	X	X
2318	X	X	X	X	X	X	X	X
2388	X	X	X	X	X	X	X	X

Msp I sites	haplotypes A	B	C	D	E	F
65	X	X	X	X	X	X
241	O	O	O	O	O	X
330	O	O	X	X	O	O
766	X	X	X	X	X	X
1259	O	X	X	O	O	X
1390	X	X	X	X	X	X
1535	X	X	X	X	O	X
2226	X	X	X	X	X	X
2403	X	X	X	X	X	X

Rsa I sites	haplotypes A	B	C	D	E
293	O	O	O	O	X
507	O	O	X	X	O
588	O	O	O	O	X
761	X	X	X	X	X
950	O	O	O	O	X
1000	X	X	X	X	O
1071	X	X	X	X	O
1263	O	O	O	O	X
1308	O	X	O	X	X
1358	X	X	X	X	X
2164	O	O	O	O	X

Sty I sites	haplotypes A	B	C	D
295	X	O	X	X
982	O	O	O	X
1748	O	O	X	O
2053	O	X	O	O
2254	X	X	X	X

Variation in yellowfin tuna (*Thunnus albacares*) catches related to El Niño-Southern Oscillation events at the entrance to the Gulf of California

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Abstract—Fishery catch data on yellowfin tuna (*Thunnus albacares*) were examined to study the effects of El Niño events between 1990 and 1999 for an area in the northeastern tropical Pacific (18–24°N, 112–104°W). The data were extracted from a database of logbook records from the Mexican tuna purse-seine fleet. Latitudinal distribution of the catches increased from south to north for the 10-year period. Highest catches and effort were concentrated between 22°N and 23°N. This area accumulated 48% of the total catch over the 10-year period. It was strongly correlated with El Niño-Southern Oscillation (ENSO) events. At least two periods of exceptionally high catches occurred following El Niño events in 1991 and 1997. Peaks of catches were triggered by the arrival of positive anomalies of sea surface temperature (SST) to the area. A delay of two to four months was observed between the occurrence of maximum SST anomalies at the equator and peaks of catch. Prior to these two events, negative SST anomalies were the dominant feature in the study area and catch was extremely low. This trend of negative SST anomalies with low catches followed by positive SST anomalies and high catches may be attributed to northward yellowfin tuna migration patterns driven by El Niño forcing, a result that contrasts with the known behavior of decreasing relative abundance of these tuna after El Niño events in the eastern Pacific. However, this decrease in relative abundance may be the result of a local or subregional effect.

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The entrance to the Gulf of California (from 18°N to 24°N and 104°W to 112°W) is located in the convergence zone of the North Pacific Gyre, where the California Current separates from the coast to feed the North Equatorial Current. It has a complex hydrographic structure due to the confluence of different water masses (Roden and Groves, 1959; Roden, 1972; Álvarez-Borrego and Schwartzlose, 1979; Bray and Robles, 1991; Torres-Orozco, 1993). This region is highly responsive to the El Niño phenomenon. Its main response is characterized by positive sea level anomalies, warming of the upper layer, and a general alteration of water current patterns (Baumgartner and Christensen, 1985; Robles and Marinone, 1987; Torres-Orozco, 1993; Lavín et al., 1997; Ortega-García et al., 1999; Trasviña et al., 1999; Castro et al., 2000; Bernal et al., 2001).

High abundance of yellowfin tuna (*Thunnus albacares*; YFT) is reported in the study area (Allen and Punsly, 1984; Castro-Ortiz and Quiñones-Velásquez, 1987; Muhlia-Melo, 1993; Ortega-Garcia, 1998). However, studies about the YFT interaction with the physical environment of the Mexican Pacific are lacking. Blackburn (1965, 1969) considers that the abundance of the YFT correlates with sea surface temperature (SST) in the range of 20°C to 30°C, but it can also be present in regions having temperatures between 18°C and 31°C. Blackburn (1969) considered 30°C as an optimal estimate of the maximum temperature of occurrence of YFT. Similarly, Ortega-García (1998) reported that YFT are distributed in regions where SST ranges from 17°C to 31°C and that they are frequently observed in waters close to 28°C. Other studies such as that of Laevastu and Rosa (1963) and Castro-Ortiz and Quiñones-Velasquez (1987) have indicated that YFT are found at concentrations favorable to commercial fisheries in regions where the SST ranges from 20°C to 28°C. In summary, YFT catches are historically reported to

occur at SST values ranging from 12° to 30°C, and favorable catches are reported between 17° and 25°C (Lehodey[1]). More recently, Bautista-Cortes (1997) has studied the relationships between varying vertical temperature structure and YFT catches in the Mexican Pacific to 140° longitude W, finding that the depth of the 23°C isotherm is related to catch and that the shallower the isotherm, the larger the tuna catches.

Castro-Ortiz and Quiñones-Velasquez (1987) reported reduced catches of YFT during the 1982–83 El Niño events in the northeastern Pacific, in contrast to the following 1984–85 seasons. The 1982–83 El Niño event was reported to have caused the largest decrease in the availability-vulnerability index ($AVI=CPUE/(1-SSR)$; where SSR is the successful-set ratio and $CPUE$ is catch per unit of effort; IATTC[2]). This finding is consistent with the resource being less susceptible to fishing in the northeastern Pacific during an intense El Niño. Lehodey[1] analyzed data of purse-seine and longline CPUE of skipjack (*Katsuwonus pelamis)*, yellowfin (*Thunnus albcares*), bigeye (*Thunnus obesus*), and albacore (*Thunnus alalunga)* tunas during El Niño and La Niña events, from 1982 to 1998 for the whole tropical Pacific Ocean (20°S to 20°N). He concluded and supports the hypothesis that ENSO affects the recruitment of skipjack, yellowfin, and bigeye tunas positively in the western Pacific. A direct positive effect related to the vertical change in the thermal structure during El Niño increased purse-seine catches of yellowfin in the western Pacific. He suggested two theoretical cases be considered for yellowfin and bigeye in the eastern Pacific: either a positive La Niña effect on the recruitment and catchability, or conversely a positive El Niño effect on recruitment and catchability. His statistical analysis did not identify the direct effects of ENSO on catchability for yellowfin and bigeye tunas. He suggested the need for additional analyses in the eastern Pacific region and for comparisons with independent results from modeling and observations to confirm the preliminary results. Lu et al. (2001) analyzed catches of YFT and bigeye tuna caught by the longline fleet of the tropical Pacific Ocean. They concluded that high hook rates for both species were mostly associated with regions where SST increased during El Niño or La Niña years. During La Niña episodes, YFT populations appear to undergo a poleward displacement thus shifting both north and south of the equator. During El Niño events YFT populations in the equatorial Pacific are found where SST anomalies are higher, in the central and eastern equatorial Pacific.

For the region of interest, Ortega-García et al. (1999) studied the impact of the ENSO during the 1997–98 El Niño events in comparison to the 1996–97 non El Niño year within the eastern Pacific Mexican purse-seine tuna fishery. They reported that during the first months of the ENSO (July–December 1997) of the El Niño event, the extent of the YFT catches was higher in oceanic waters than the observed catches during a non El Niño year in these areas for the same period. A decline of effort was observed along the coast of Baja California and inside the Gulf of California. In contrast, during the second part of the ENSO El Niño 1997–98 (January–June 1998), an increase of effort was observed along the coasts of Baja California and inside the Gulf of California; however, a decrease of effort was observed in oceanic areas.

The relative abundance of YFT in the eastern Pacific is reported to diminish during El Niño events; however, as supported by the IATTC[2] report, the YFT vertical displacement during El Niño events generates a diminishing fishing effort for the purse-seine fleet in traditional catch areas. As a consequence, this has led to a good recruitment and greater yield-per-recruitment. Large recruitments after El Niño events are reported to occur in the eastern Pacific. Joseph and Miller[3] analyzed YFT catch data from the eastern Pacific for a 22-year period and found positive anomalies in tuna recruitment after El Niño events. Large recruitments were observed in years 1971, 1974, 1978 and 1985, all of which were preceded by the El Niño events of 1969, 1972, 1976, and 1983, respectively.

The objective of the present study was to examine the effect of the ENSO El Niño and La Niña events for a 10-year period (1990–99) on YFT catches in the entrance to the Gulf of California, an area of importance for the industry and for the ecology of the resource in the eastern Pacific.

Materials and methods

The classification of El Niño intensities was taken from the Climate Prediction Center (NOAA[4]). The process of classification takes into account re-analyzed SSTs produced at the National Centers for Environmental Prediction (NCEP), at the Climate Prediction Center, and at the United Kingdom Meteorological Office.

Environmental data in the form of monthly means of sea surface temperature anomalies (SSTAs) from 1990 to 1999 were extracted from the NCEP monthly SSTA data-

[1] Lehodey, P. 2000. Impacts of the El Niño Southern Oscillation on tuna populations and fisheries in the tropical Pacific Ocean. Working Paper. RG-1, 32 p. Oceanic Fisheries Programme, Noumea, New Caledonia, Secretariat of the Pacific Community. http://www.spc.org.nc/OceanFish/Html/SCTB/SCTB13/rg1.pdf [accessed on 20 February 2002].

[2] IATTC (Inter American Tropical Tuna Commission). 1989. Annual Report of the Inter-American Tropical Tuna Commission, 1988. Annu. Rep. IATTC, 288 p. http://www.iattc.org/PublicationsSPN.htm [accessed on 11 March 2002].

[3] Joseph, J., and F. R. Miller. 1988. El Niño and the surface fishery for tunas in the eastern Pacific. *In* Proceedings of tuna fish. res. conf., p. 199–207. Japan Fish. Agency-Far Seas Fish. Res. Lab. Muguro Gyogyo Kyogikai Gjiroku, Suisancho-Enyo Suisan Kenkyusho.

[4] NOAA (National Oceanic and Atmospheric Administration). 2001. Cold and warm episodes by season. Website: http://www.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/ensoyears.html [accessed on 10 January 2002].

base (IRI[5]). The SST fields were blended from ship, buoy, and bias-corrected satellite data. A description of this procedure can be found in Reynolds and Smith (1994). SSTA data for El Niño region 3 (NIÑO3, from 150°W to 90°W and from 5°N to 5°S) were obtained from Climate Prediction Center databases (CPC[6]).

Yellowfin tuna catch data were obtained from the database of the ATUN (tuna) Project of CICIMAR (the Interdisciplinary Research Center of Marine Science of the National Polytechnic Institute in La Paz, Mexico). This database has information on daily fishing activities for about 80% of the Mexican purse-seine fleet operating in the eastern Pacific. The data used in the present study include carrying capacity, catch-by-species, location, and school type (YFT associated with dolphins; YFT associated with floating objects; and free-swimming YFT), from 1990 to 1999, comprising 11,690 records. Total distribution of the sets made by the Mexican purse-seine fleet from 1990 to 1999 at the entrance of the Gulf of California is shown in Figure 1.

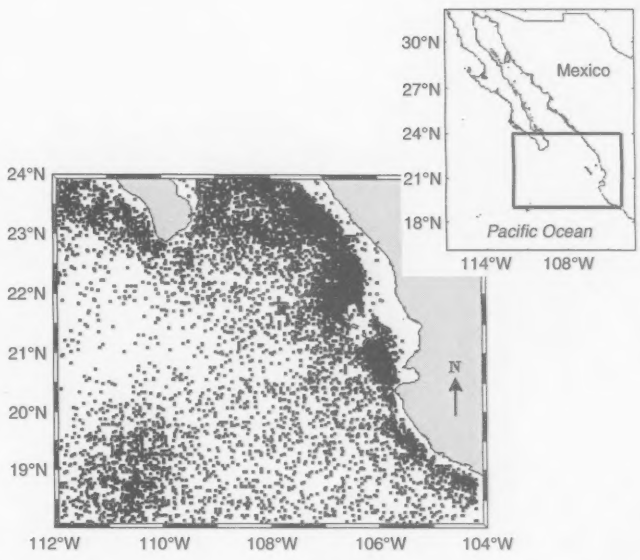

Figure 1

Total distribution of the sets made by the Mexican purse-seine fleet during 1990 to 1999 at the entrance to the Gulf of California. Catch data for yellowfin tuna (*Thunnus albacares*) were obtained from the CICIMAR (the Interdisciplinary Research Center of Marine Science of the National Polytechnic Institute in La Paz, Mexico).

Interannual variation

In order to study annual variation, total catches of YFT of the purse-seine fleet within the study area were accumulated by year. A cross-correlation analysis was applied to obtain information on lags between SST anomalies and YFT catches.

Latitudinal stratification

In order to analyze the latitudinal catch variation of YFT within the study area, catch data for the period 1990–99 were accumulated in six latitudinal bands of one degree from 18°N to 24°N at the entrance of the Gulf of California. Longitudinal limits extended from the coastal line of the Mexican continent (from 104°W to 112°W).

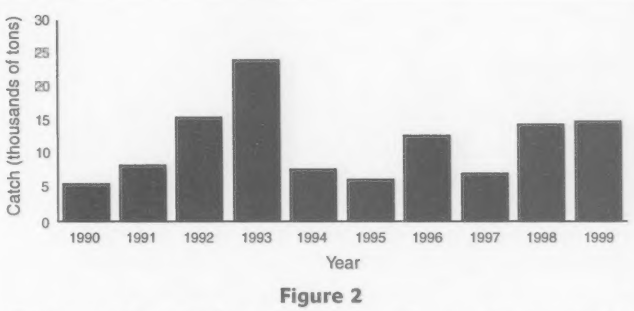

Figure 2

Total catch for yellowfin tuna (*Thunnus albacares*) by year during 1990–99 in the study area.

Results

Interannual variation in YFT catch

The catch of YFT in the study area showed high interannual variation (Fig. 2). This high variation showed substantial increments, 85% from 1991 to 1992, 54% from 1992 to 1993, 102% from 1995 to 1996, and 102% from 1997 to 1998. In particular, the sum of the two El Niño years in the record (1991–93, 1997–98) represents 59.5% of the total catches over the 10-year period.

Latitudinal variation in YFT catches

Figure 3 shows the variation of catch and effort (in sets) from 1990 to 1999. Vertical bars include catches from different one-degree latitudinal range areas between 18°N and 24°N and meridional ranges between 104°W to 112°W (Fig. 1). With the exception of the southern 18–20°N area,

[5] IRI (International Research Institute for climate prediction). 2002. Sea surface temperature anomaly data. Website: http://ingrid.ldgo.columbia.edu/SOURCES/.NOAA/.NCEP/.EMC/.CMB/.GLOBAL/.Reyn_SmithOIv1/.monthly/.ssta/ [accessed on 9 February 2002].

[6] CPC (Climate Prediction Center). 2002. Monthly atmospheric and SST indices. Website: http://www.cpc.ncep.noaa.gov/data/indices/index.html [accessed on 9 February 2002].

there is a general linear increase of catch with latitude. Fishing effort was concentrated in the 22–23°N and 23–24°N areas. Both account for 48% of the total capture for the 10-year period in this region. From 18° to 21°N, catch values averaged 12.9% of the total catch.

Yellowfin tuna catches affected by ENSO events

Sea surface temperature anomalies (SSTA) were used to investigate the effect of interannual warming or cooling events on the variability of YFT catch. Anomalies for

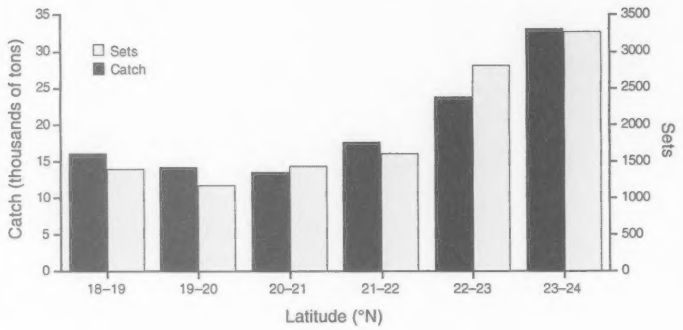

Figure 3

Latitudinal distribution of catches of yellowfin tuna (*Thunnus albacares*) for the period 1990–99.

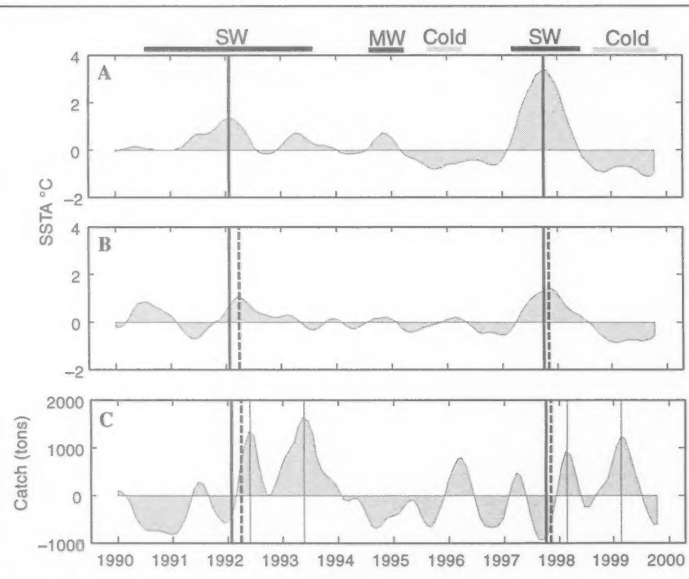

Figure 4

Sea surface temperature anomalies (SSTAs) in the El Niño region 3 (**A**), SSTAs in the study area (**B**) and catches of yellowfin tuna (*Thunnus albacares*) (**C**). Bars at the top show the equatorial central Pacific episodes as compiled by the Climate Prediction Center (see "Methods" section): Strong warm (SW) peaks of catch are indicated by thin vertical lines. Thick vertical lines indicate maximum SST anomalies in NIÑO3 (A). Dashed vertical lines indicate peaks of SSTA in the area of study (B). Moderate warm (MW) and cold episode. The maximum intensity of the warm episodes is marked with dashed lines (B and C).

the entrance to the Gulf of California were calculated between 22°N and 23°N and 103°W to 112°W. These anomalies were extracted from the Reynolds and Smith (1994) monthly 1-degree SSTA climatology. This variable was then compared to the catch of YFT. We did not examine SSTAs south of 21°N because they fall within the Mexican Warm Pool (Trasviña et al., 1999), where persistently high SSTs (above 27°C all year in the warm pool region) mask the propagation of warm and cold signals from the Equator. The 10-year time series (1990 to 1999) was "detrended" and filtered to eliminate periods shorter than three months, as described in Godin (1972).

SSTAs in the NIÑO3 (Fig. 4A) showed cold and warm conditions associated with five identifiable events. These events were the following: 1) warm El Niño events in 1991–92 (strong warm, SW), 2) in 1994–95 (moderate warm, MW), and 3) in 1997–98 (strong warm, SW); 4) moderately cold La Niña events that took place in 1995–96 and 5) in 1998–99. Strong warm events are marked with a black line located at each respective maximum SSTA (Fig. 4). The 1997–98 was the strongest warm episode in the 1990–99 decade.

The 1991–92 and 1997–98 SSTAs in El Niño region 3 were higher, by more than 1°C, than the SSTAs in the study area (Fig. 4, A and B). Within the 10-year period, near-normal conditions occurred from 1993 to 1996. The coldest and most persistent event of this decade took place in the study area from July 1998 to December 1999, i.e., during a moderate La Niña event.

Significant interannual variability of YFT catches was observed during the 10-year period in the study area (Fig. 4C). This graph shows the time series of monthly values of YFT catch anomalies related to the average for the 10-year period. Five exceptionally high catch events in years 1992, 1993, 1996, 1998, and 1999 were observed. These years account for 70% of the total capture during the 10-year period.

Several qualitative and quantitative features were apparent from these results:

1 Catch peaks in 1992 and 1998 occurred 3 (r=0.73) and 4 (r=0.64) months, respectively, after the onset of an El Niño event at the equator (time span between thin and thick lines) (Fig. 4, A and C).
2 High catches of YFT at the entrance of the Gulf of California in 1992 and 1998 occurred 2 (r=0.71) and 3 (r=0.73) months, respectively, after the SST anomaly signal reached the study area (time span between dashed and thin lines) (Fig. 4, B and C).
3 Higher catches of YFT occurred during the spring following an El Niño winter. These were observed in the spring of 1992 and 1998 (thin lines; Fig. 4C).
4 Peaks of YFT catch were also observed in 1993 and 1999. These occurred one year after the El Niño event. The 1993 peak took place in nearly normal SST conditions, whereas the 1999 peak occurred during negative SST anomalies (La Niña event, Fig. 4, B and C). These peaks of YFT catch were higher than the ones recorded in the previous year during the El Niño event 1991–92 and during 1997–98.

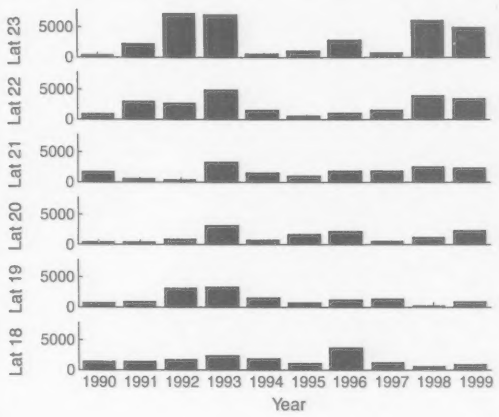

Figure 5

Annual catches (in tons) of yellowfin tuna (*Thunnus albacares*) in latitudinal bands within the area of study for the period 1990–99.

5 The 1996 peak seemed not to be related to the variability of SST anomalies alone.
6 There was a delay from one to two months between the mature phase of the El Niño event at the equator and the presence of high SSTAs in the study area (time span between thick and dashed lines in Fig. 4, A and B).

Annual YFT catches of stratified latitudinal bands within the study area are shown in Figure 5. A progressive increase in YFT catches from southern to northern latitudes was observed in years 1993 and 1999. This progressive increase may be indicative of a northward movement of the resource. This phenomenon has been observed in the western tropical Pacific Ocean during La Niña events where YFT have dominated the longline catch (Lu et al., 2001).

Discussion

Catch affected by the onset El Niño

Cross-correlation between the onset of El Niño at the equator in 1991–92 and 1997–98 with catch peaks of YFT in the study area showed a delay of three and four months respectively. Catch peaks of YFT at the entrance of the Gulf of California in 1992 and 1998 occurred two to three months after the SSTA signal reached the study area. These results are similar to those found by Lehodey[1] in the western and central regions of the Pacific Ocean, where rising (deepening) of the mixed-layer depth related to El Niño (La Niña) was associated with an increase in the pole-and-line and purse-seine CPUE of YFT and where there

was a concomitant delay of two to three months in this increase.

Catch of YFT in relation to recruitment

Recruitment explains most of the YFT catch fluctuations in relation to El Niño events at the entrance of the Gulf of California. A recurrent pattern in the time-series of the catch revealed a peak of catch fourteen (1993) to twelve (1999) months following an El Niño event. A similar result has been observed in a cross-correlation between the Southern Oscillation index (SOI) and the long-line series of catch data for yellowfin tuna; a positive correlation was found in the eastern Pacific region after a lag of fourteen months (Lehodey[1]). This finding supports the hypothesis of a recruitment base (age-effect) for yellowfin because at 14 months YFT reach 75 cm, corresponding to the first age class recruited to the long-line fishery. These results are also consistent with those from previous analyses of time-series of catches of YFT in the eastern Pacific, namely by Joseph and Miller[3] and the IATTC[2]. Therefore, recruitment can be the major explanation of the YFT catch fluctuations in relation to El Niño events at the entrance of the Gulf of California.

Catch distribution of YFT related to ENSO episodes

For the long-line fishery in the tropical Pacific Ocean, areas with significant higher hook rates of YFT during El Niño years are located east of 150°W within tropical waters of the central eastern Pacific (Lu et al., 2001). Conversely, higher hook rates occur during La Niña episodes in areas where SSTs rise during El Niño events. These two findings may provide two possible reasons for the change in hook rates: the expansion of optimal habitat and the change in vulnerability of the resource to the fishing gear during the ENSO episodes.

Conclusions

At the entrance of the Gulf of California, an El Niño event is associated with an increase in the purse-seine catch of YFT after two to three months, when SSTAs reach the study area. Similarly, this increase is delayed three to four months after the onset of El Niño at the equator.

A positive correlation of El Niño on YFT catch at the entrance of the Gulf of California after a twelve to fourteen month delay supports the hypothesis that the ENSO affects recruitment of YFT. This correlation seems to be independent of the thermal structure during the recruitment phase. In 1993, normal conditions in SST were present, whileas in 1999 La Niña was observed.

A northward displacement of YFT seems to occur at the entrance of the Gulf of California twelve to fourteen months after El Niño events. Lower catches in southern latitudes were recorded, whereas higher catches were recorded in northern latitudes. Similar results have been observed in the central Western Pacific for the long-line fishery (Lu et al., 2001).

Forecasting catch within the study area may give us the ability to reliably develop predictions of catch following El Niño or La Niña events.

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Estimating the encounter rate of Atlantic capelin (*Mallotus villosus*) with fish eggs, based on stomach content analysis

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Abstract—The number of pelagic fish eggs (cod and cunner) found in stomachs of capelin (*Mallotus villosus*) sampled in coastal Newfoundland was used to estimate the encounter rates between capelin and prey, and thus the effective volume swept by capelin. Fish eggs were found in 4–8% of capelin stomachs, represented an average of 1% of prey by numbers, and their abundance increased as relative stomach fullness decreased. The average number of eggs per stomach doubled for each 5-cm increase in length of capelin. The effective volume swept for eggs by capelin ranged from 0.04 to 0.84 m^3/h—a rate that implies either very slow capelin swimming speeds (<1 cm/s) or that fish eggs are not strongly selected as prey. The predation rate estimated from stomach contents was higher than that predicted from laboratory studies of feeding pelagic fish and lower than that predicted by a simple foraging model. It remains uncertain whether capelin play an important regulatory role in the dynamics of early life stages of other fish.

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Capelin (*Mallotus villosus* (Müller, 1776)) is a key species in Arctic ecosystems, serving as a major prey for top predators, from fish to birds and whales (Akenhead et al., 1982; Gjøsæter, 1998; Vilhjálmsson, 2002). Off the coast of Newfoundland and Labrador (eastern Canada), capelin are also the dominant consumers of secondary production (O'Driscoll et al., 2001), as they are in other ecosystems (Skjoldal and Rey, 1989; Hassel et al., 1991). They feed on a wide variety of zooplankton taxa, copepods and euphausiids being the dominant prey (Vésin et al., 1981; Panasenko[1]; Huse and Toresen, 1996; Assthorsson and Gislason, 1997; O'Driscoll et al., 2001). The choice of prey shows some degree of size-dependency; copepod size increases with increasing size of capelin, and euphausiids become more frequent prey items as fish size increases. In most studies of the feeding habits of capelin, fish eggs and larvae have been found to have been eaten by capelin, although these prey types generally represent a minor portion of the diet, occurring in less than 5% of stomachs (Huse and Toresen, 1996; O'Driscoll et al., 2001). Because capelin are dominant consumers of zooplankton in many of the ecosystems in which they are present, their overall impact on the survival of pelagic fish eggs and larvae may be significant depending on their encounter rates with these fish eggs and larvae.

There is evidence that capelin can have a significant impact on the survival of ichthyoplankton through predation on pelagic fish eggs and larvae. In a series of patch studies, Pepin et al. (2002) found that the mortality rate of radiated shanny (*Ulvaria subbifurcata*, Storer 1839) larvae increased in direct relation to hydroacoustic estimates of capelin abundance; this loss could be attributed to predation by capelin on fish larvae. In a study of two coastal areas, differences in the regional patterns of selective loss of radiated shanny larvae, derived from sequential observations of the distribution of growth rates, were strongly associated with differences in the spatial distribution and abundance of capelin (Baumann et al., 2003; Pepin et al., 2003). However, the overall impact of capelin on fish eggs and larvae has been difficult to estimate (Paradis and Pepin, 2001). Much of the basic foraging ecology is unknown (e.g., predator's reactive distance, behavior of both predator and prey, and selection of prey) and laboratory experiments can only provide a rough approximation of encounter rates between capelin and their prey (Paradis et al., 1996).

There have been few studies to provide an estimate of the contribution of predation by planktivorous fish on the overall mortality rates of fish eggs or larvae (Hunter and Kimbrell, 1980; Cowan et al., 1999; Köster and Möllmann, 2000; Munk, 2002; Pepin et al., 2002). Population studies have shown that planktivorous fish can play an important role in regulating the stock-recruitment relationship of other species (Swain and Sinclair,

[1] Panasenko, L. D. 1984. Feeding of Barents Sea capelin. ICES CM 1984/H:6, 16 p.

Table 1

Location, average zooplankton density (all organisms), average fish egg density, and number of capelin (*Mallotus villosus*) stomachs collected at each sampling site.

Site	Latitude	Longitude	Average zooplankton density (/m^3)	Average fish egg density (/m^3)	No. of stomachs colleccted at each site					
					00:01–04:00	04:01–08:00	08:01–12:00	12:01–16:00	16:01–20:00	20:01–24:00
1	47°44′N	53°40′W	1255	0.094	50	45	—	—	—	—
2	48°18′N	53°16′W	319	0.91	35	50	17	30	18	48
3	47°44′N	53°38′W	2766	0.23	—	100	22	—	—	—
4	48°02′N	53°36′W	210	0.29	99	50	99	50	9	49
5	48°19′N	53°15′W	3096	0.58	51	49	44	49	49	47

2000; Köster et al., 2001; Garrison et al., 2002). Individual-based models have often served as a tool to infer the potential effect of predators on early life stages (Cowan et al., 1999; Paradis et al., 1999; Werner et al., 2001) where the likelihood of a prey being eaten by a predator is the product of the probabilities of encounter, attack, and capture. Although the latter two components of the predation process have been estimated in the laboratory for a number of species (see Paradis et al., 1996, for a review), encounter rates are often modeled as the product of predator's swimming speed and reactive distance with limited substantive data to support the overall estimate. Furthermore, encounter rates are assumed to occur randomly and thus follow a Poisson distribution; yet there have been few attempts to validate this assumption (but see Huse and Toresen, 2000). Given that encounter rates represent a key parameter required to estimate the potential impact of predators on early life stages, it is surprising that knowledge of this element in the field is so limited.

The goal of the present study was to estimate effective encounter rates of adult capelin with fish eggs and larvae in coastal Newfoundland. The approach was to obtain a high number of specimens from a series of intensive diurnal collections to provide an accurate estimate of the probability distribution of prey numbers from which encounter rates could be derived. The primary focus of this analysis was on the presence and frequency of occurrence of fish eggs as prey for capelin, because they persist longer in stomachs than do fish larvae (Hunter and Kimbrell, 1980; Folkvord, 1993). I used estimates of prey availability from plankton samples combined with the frequency of occurrence of eggs in stomachs, in relation to the size of capelin and time of day, to estimate effective encounter rates (volume searched) by capelin. Fish eggs are well suited to this objective: they are completely passive and thus the probabilities of their avoidance of both plankton nets and predators can be considered as nil. It is thus possible to measure accurately their abundance in the field, given appropriate choice of mesh size, and their numbers in predator stomachs can provide a measure of the effective volume searched, which is the product of the probabilities of encounter and attack.

Materials and methods

The study was conducted in Trinity Bay, Newfoundland, Canada, between 17 and 31 July 2000, as part of a larger program dealing with the dynamics of ichthyoplankton (Baumann et al., 2003). Five sites were selected at which to conduct diurnal sampling of capelin based on the observation of high juvenile and adult capelin densities from hydroacoustic returns. Sampling was conducted within a 3-km radius of each site and at 4-hour intervals over a single twenty-four hour interval. Each site was treated as an independent observation of the diurnal feeding patterns of capelin. As a result of poor catches, sampling was stopped after two sampling periods at the two sites located at the head of the bay (Table 1).

Capelin were sampled using an international young gadoids pelagic trawl (IYGPT) mid-water-trawl towed in a single oblique haul from the surface to a 100-m depth. Each tow lasted approximately 30 minutes. Up to 50 juvenile and adult capelin were taken at random from the catch. When capelin were highly numerous, sample size was increased to 100. For each specimen, total length (TL) was measured to the nearest mm, the entire intestinal tract was removed by cutting the oesophagus as close to the mouth as possible, and the tract was then preserved in individual numbered vials containing 5% formaldehyde. A total of 1052 specimens were obtained from these collections. After each trawl haul, a plankton sample was taken by using a 60-cm bongo net (fitted with 333-ìm mesh net and flowmeters) towed obliquely from the surface to 100 m along the same path as the mid-water-trawl, and the sample was preserved in 2% formaldehyde solution.

Stomachs were processed by slitting them open and scraping out the contents onto preweighed aluminium weight boat. The contents were blotted dry to remove all excess liquid and weighed to the nearest 0.01 mg by using a Cahn microbalance. Stomach contents from each capelin specimen were placed into individual petri dishes and examined for the presence, number, and taxonomic identification of pelagic fish eggs only. An egg was counted only when the specimen was complete and

the chorion was relatively intact. The presence of empty chorions was noted but these were not included in the analysis because of their potentially longer residence time in the stomach of predators (Hunter and Kimbrell, 1980). As it turned out, empty chorions occurred in less than 2% of capelin with fish eggs present in their gut. For every fifth capelin specimen, all prey items were identified according to broad taxonomic groups: Copepoda, Amphipoda, Euphausia, Pteropoda, molluscan veligers, brachyuran zoeae, Larvaecea, Cladocera, Chaetognatha, Isopoda, and fish eggs and larvae. The number of individuals was determined for each taxonomic group from the entire stomach content and the total weight for each group was determined to the nearest μg by using a Cahn microbalance. This protocol was followed to provide a complete assessment of the presence of fish eggs in the gut and to provide a representative assessment of the patterns of prey composition from each site to confirm that general feeding patterns were consistent with results from previous studies. O'Driscoll et al. (2001) had performed a detailed analysis of feeding patterns of capelin in Newfoundland waters and I perceived no need or advantage in repeating the work. The complete analysis of stomach contents was performed on 218 capelin specimens.

All fish eggs and larvae were sorted from plankton samples and identified to the lowest taxonomic level possible. Because of difficulties in differentiating early developmental stages of two species groups, eggs were lumped into either of two groups: 1) CHW (namely, cod (*Gadus morhua* L.), haddock (*Melanogrammus aeglefinus* L.) and witch flounder (*Glyptocephalus cynoglossus* L.) or 2) CYT (cunner (*Tautogolabrus adspersus* L.), yellowtail flounder (*Limanda ferruginea*, Storer 1839)). Eggs were most likely those of cod, for the CHW category, and cunner, for the CYT category because only larvae of those species were found in other elements of the research program. A subsample of other zooplankton, yielding 200–300 specimens, was identified to the lowest taxonimic level possible.

For each capelin, the total gut fullness index (GFI) was estimated as $GFI=W_s/W$, where W_s=the weight of stomach contents (g); and W=is the weight of the fish (g), based on the local bias-corrected length-weight relationship ($\log_{10} W=-6.44+3.38 \log_{10} TL$, where W=weight in g and T=total length in mm; $r^2=0.98$; and the standard error of the intercept and slope are 0.049 and 0.023; Mowbray[2]).

Analysis

Stomach content composition

For each capelin on which the full stomach contents were analyzed, the number of each major prey taxon was determined. These data, arcsine-square-root transformed, were contrasted using principal component analysis in which the proportion of prey type, log-transformed capelin length and GFI were included to determine if the observations from this study were consistent with those from previous ones.

Frequency of fish eggs

The probability distribution of egg numbers per gut was contrasted with the expectations based on a Poisson process. Encounters between predator and prey can be described by using a modification of Gerritsen and Strickler's (1977) equations where

$$\lambda = \pi \cdot R^2 \cdot A_{prey} \cdot \sqrt{u^2 + v^2}, \quad (1)$$

where R = the reactive distance (m);
A_{prey} = the density of prey (/m^3); and
u and v = the swimming speeds of predator and prey (m/h), which yields an encounter rate λ (per h).

Huse and Toresen (2000) used laboratory estimates of the various parameters to estimate encounter rates between juvenile herring (*Clupea harengus* L.) and capelin larvae. Instead of using their approach, I chose to reduce Equation 1 to its simplest form, such that

$$\lambda = K \cdot A_{prey}, \quad (2)$$

where K = the effective volume swept (m^3/h) during the period during which eggs will remain discernible in the gut of capelin; and
A_{prey} = the observed density of eggs (/m^3) in the water column from plankton samples.

The resulting probability of finding N prey in the gut of capelin then becomes

$$P(N) = \frac{\lambda^N}{N!} \cdot e^{-\lambda}, \quad (3)$$

where λ = the mean encounter rate.

Mean number of eggs per stomach (N), taken as a measure of encounter rates for each four-hour sampling interval, was estimated by fitting a generalized linear model with Poisson error structure using a log link function by maximum likelihood (GENMOD procedure, vers. 8, SAS Inc., Cary, NC) to the data from all 4-hour sampling intervals (T) from all locations, with TL of individual capelin as a linear covariate.

$$f(N) = b_0 + b_1 T + b_2 TL. \quad (4)$$

The value of N reported in the "Results" section is the least squares adjusted mean, which represents the value of the average length of capelin from field collections. We attempted to include site into the analysis but the

[2] Mowbray, F. 2005. Personal commun. Fisheries and Oceans, St. John's, Newfoundland, Canada.

algorithm failed to converge because of missing observations. Effective volume swept (K) was calculated from the estimated mean number of eggs per stomach (as a measure of encounter rates) and the estimated density of eggs based on plankton samples by re-arranging Equation 2 and assuming that encounters between prey and predators were random over the top 100 m of the water column. I define K as the effective volume swept because it implicitly includes some measure of the probabilities of attack and capture. Values of the estimated volume swept were corrected for evacuation rates. Hunter and Kimbrell (1980) found that the evacuation rate of northern anchovy (*Engraulis mordax*, Girard 1854) feeding on fish eggs at 15°C was ~50%/h based on an exponential model (exponential rate of 0.7/h), which is appropriate for microphagic fish (Temming et al., 2002). Based on the duration of our tow and the handling time to process the samples, the observations of stomach content from this study represent 50% of the eggs consumed during the last hour before capture. Hunter and Kimbrell's (1980) estimate of the evacuation rate is approximately twice that obtained in field studies by Arrhenius and Hansson (1994) and Darbyson et al. (2003) for herring (*C. harengus* L.) feeding primarily on copepods. However, the estimates from the latter two studies were based on total gut fullness and not on the disappearance of specific food items, such as fish eggs. As a result, Hunter and Kimbrell (1980) provided the only current measure of the rate of disappearance of eggs in the stomachs of planktivorous fish. Increases or decreases in evacuation rates because of inaccuracies in our knowledge of the rate of disappearance of eggs from stomach contents will result in corresponding changes in the estimate of the effective volume swept. By using the mean number of eggs per stomach as an estimator of the effective volume swept, the probabilities of attack and capture within the reactive distance are assumed to be 100%. The resulting value of the effective volume swept represents an average over the top 100 m of the water column, the depth range over which we sampled both capelin and zooplankton and over which I assume that the encounters are random. Ideally, one would want to obtain a depth-stratified measure of stomach contents, and prey availability and overlap between prey and predator, such as has been inferred in the Baltic Sea (Köster and Möllmann 2000; Köster et al. 2001), but this was not possible in the present study because there were insufficient data on the vertical distribution of capelin.

Results

Capelin used in this analysis ranged from 53 to 195 mm TL (mean=122 mm; median=130 mm). Average zooplankton concentrations ranged from 210 to 3096 organisms/m^3. In most samples, the copepods *Calanus finmarchicus* and *Pseudocalanus* sp. and the larvacean *Fritillaria borealis* (Lohmann 1896) represented 60–80% of the zooplankton by number, based on samples from bongo nets. In both species of copepods, copepodite stages II and III were most abundant. A finer mesh net would have revealed that *Oithoina similis* was also

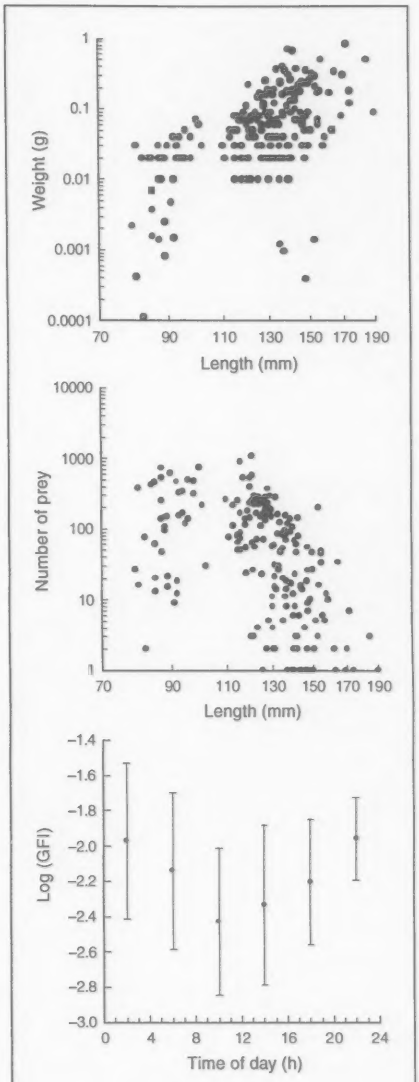

Figure 1

Weight of stomach contents (upper graph) and number of prey per stomach (center graph) in relation to capelin (*Mallotus villosus*) length, for specimens with complete analysis of contents, and average and standard deviation of the log-transformed gut fullness index (GFI) in relation to time of day (lower graph), based on all fish sampled. Statistics were calculated for all samples collected within each 4-hour time interval.

Table 2

Frequency of occurrence (number of capelin [*Mallotus villosus*] for which complete stomach analysis was performed) and average relative proportion of prey (by number) for the dominant prey categories found in July 2000. The total number of capelin examined for complete stomach analysis was 218. There were 12 fish with empty stomachs.

Prey type	Frequency of occurrence	Relative proportion
Copepoda	176	0.77
Euphausia	50	0.066
Amphipoda	43	0.088
Pteropoda	22	0.010
Larvacea	21	0.032
Cladocera	17	0.022
Fish eggs	16	0.010
Chaetognatha	4	<0.001
Mollusca veliger	3	0.001
Brachyura zoea	2	0.005
Isopoda	1	<0.001

Table 3

Results of the principle components (PC) analysis of proportion of prey categories from capelin stomachs. Values in parentheses along the topmost row represent the proportion of the variance explained by each principle component. Column values represent the average loading of each variable included in the analysis. *GFI*= gut fullness index.

Variables	PC 1 (17%)	PC 2 (12%)	PC 3 (10%)
Ln (*capelin length*)	0.494	−0.068	−0.144
Ln (*GFI*)	0.166	−0.575	0.325
Copepod	−0.598	−0.276	−0.081
Cladocera	−0.035	0.315	0.593
Pteropoda	0.044	−0.131	−0.050
Amphipoda	0.490	−0.017	−0.026
Euphausia	0.330	−0.153	0.122
Cheatognatha	−0.066	−0.083	−0.110
Mollusca	−0.017	0.260	0.550
Isopoda	−0.023	0.003	−0.173
Larvacea	−0.011	0.440	−0.065
Brachyura	0.038	0.280	−0.310
Fish eggs	0.106	0.340	−0.232

numerically important in the area (Pepin and Maillet[3]), but O'Driscoll et al. (2001) did not find this species in any of their stomach samples despite its importance in the regional plankton community. Fish eggs represented an average of 0.13% of the total zooplankton community among all sites sampled (Table 1).

Only 12 of 218 capelin stomachs, for which complete stomach analysis was performed, were found to be empty. The weight of prey in capelin stomachs increased with increasing length but the number of prey decreased with increasing length, indicating that the average size of prey increased with capelin length (Fig. 1). Stomach fullness increased at sunset, peaked shortly after midnight, and then gradually decreased during the remainder of the night and day (Fig. 1). Copepods were the only prey in 73 of the remaining 206 capelin. Euphausiids and amphipods were the next most abundant prey item, found in approximately 25% of capelin stomachs and representing on average 6.5% and 8.8% of prey by number (Table 2). Pteropods, larvaceans, cladocerans and fish eggs were present in approximately 10% of capelin stomachs and represented ~1–3% of prey by numbers (Table 2). Other prey taxa were found in a few capelin stomachs. Principal components analysis showed that the proportion of euphausiids and amphipods in stomachs increased with capelin length, whereas copepods were more frequent in smaller fish, as indicated by the first principal component (Table 3). Other prey categories, particularly fish eggs and larvaceans, were generally more likely to occur in capelin stomachs when they were relatively empty, as indicated by the second principle component (Table 3). Finally, the third principal component indicated that fish eggs appeared to be less important in areas where mollusks and Cladocera were more frequent in the diet of capelin—a finding that possibly reflects the influence of water masses in which coastal rather than shelf zooplankton were dominant (Table 3). These observations of prey composition were generally consistent with those of O'Driscoll et al. (2001).

When we consider all stomachs analyzed for fish eggs, the overall frequency of occurrence of fish eggs was 4.3% (46 of 1052 stomachs). This is less than the value (7.9% of stomachs with prey) obtained from those stomachs with complete analysis of prey composition. The frequency distribution of fish eggs was well described by a Poisson distribution (Fig. 2). The majority of fish eggs (89%) were of the CHW complex and the remainder (11%) were of the CYT complex. The generalized linear model revealed that there was a significant effect of time of day (Table 4), and the greatest number of fish eggs in capelin stomachs were found between 20:00 and 24:00, following sunset, and the lowest numbers were found between 00:00–04:00 (Fig. 3). There was approximately a 20-fold difference in the least squares

[3] Pepin, P., and G. L. Maillet. 2002. Biological and chemical oceanographic conditions on the Newfoundland Shelf during 2001 with comparisons with earlier observations. Canadian Science Advisory Secretariat, Research Document 2001/073, 60 p. [Available from http://www.dfo-mpo.gc.ca/csas/ or Canadian Science Advisory Secretariat, Department of Fisheries and Oceans, 200 Kent Street, stn. 12032, Ottawa, Ontario, Canada K1A 0E6.]

Table 4

Analysis of deviance for the effects of time of day and capelin TL with Poisson error based on a generalized linear model. The effect of time of day was based on grouping data into 4-hour classification periods. Probability values were calculated by using a χ^2 function.

			Difference		
Variable	Residual degrees of freedom (df)	Residual deviance	df	Deviance	*P*
	1052	365.9			
Time of day	1046	321.7	6	44.2	<0.001
Length	1045	317.2	1	4.5	<0.05

mean estimate of the number of eggs per stomach over a diurnal cycle. There was a significant and positive effect due to capelin length (χ^2=4.5, P<0.05; Table 4) which would yield a twofold increase in the mean number of eggs per stomachs for each 5-cm increase in capelin length. This finding may indicate that smaller capelin are more effective predators of fish eggs, in terms of body mass, than larger individuals. There were insufficient data to obtain reliable estimates of the diurnal feeding periodicity at each site.

The overall unweighted average density of fish eggs among all sites was 0.52/m^3 (median=0.48/m^3; mean=0.53/m^3; SD=0.43/m^3; range=0.09–2.2/m^3). There were significant differences in the densities of fish eggs among sites (ANOVA $F_{4,21}$=4.56, P<0.01); a Bonferoni *post hoc* comparison revealed that site 2 had significantly higher densities than other sites, with the exception of site 5 (Table 1). However, there was no clear evidence of a diurnal pattern to the density estimates derived from plankton collections. Because there were insufficient data to obtain reliable estimates of the diurnal feeding periodicity at each site and because there was no clear evidence of a diurnal pattern in egg densities estimated from the plankton samples, I chose to use the overall unweighted average density from all collections as the estimate of A_{prey} for Equation 2.

Because it was not possible to obtain a site-specific estimate of diurnal patterns in egg consumption by capelin, the combined data on egg densities were used to estimate the effective volume swept (K). Each estimate of the mean number of eggs per stomach was taken to represent 50% of the eggs ingested in the last hour during each sampling interval (4 h) (see "Methods"). The overall daily average effective volume swept based on mean encounter rates was 0.27 m^3/h, which ranged from 0.04 m^3/h at night (00:00–04:00) to 0.84 m^3/h following sunset (20:00–24:00) (Fig. 4). Based on a reactive distance of 0.15 m (~1 body length) used by Huse and Toresen (2000) (for herring larvae from Utne-Palm, 2000), the effective volume swept estimated from the observations reported in the present study would imply that the swimming speed of capelin would be very low (≤1 cm/s) or that fish eggs are not strongly selected as prey during feeding. If on the other hand we consider modal capelin (0.13 m) swimming at 0.5–1 body length/s, the reactive distance would range from 0.024–0.034 m at the time of peak foraging activity, assuming 100% probability of attack within the reactive area. To determine the effect of variations in the density of eggs encountered by capelin, I also calculated the variability

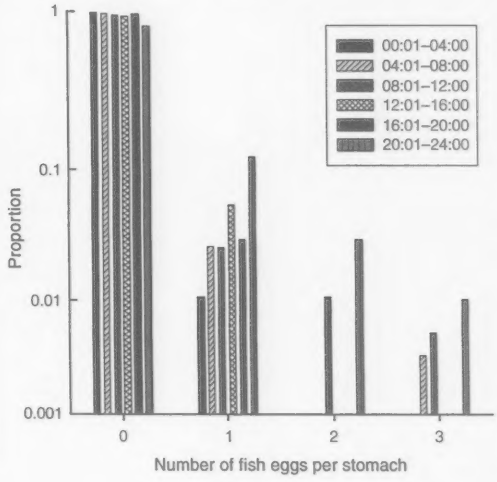

Figure 2

Bar chart of the frequency of fish eggs in capelin (*Mallotus villosus*) stomachs for each 4-hour time interval.

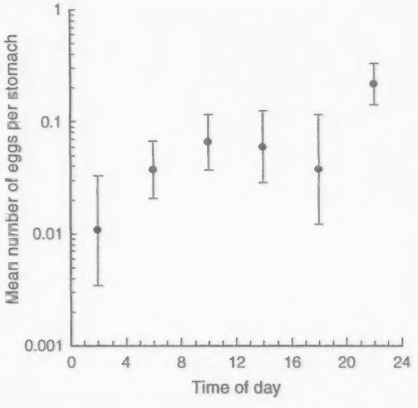

Figure 3

Least squares estimates of the mean number of fish eggs per stomach in relation to time of day. Error bars represent 1 standard error.

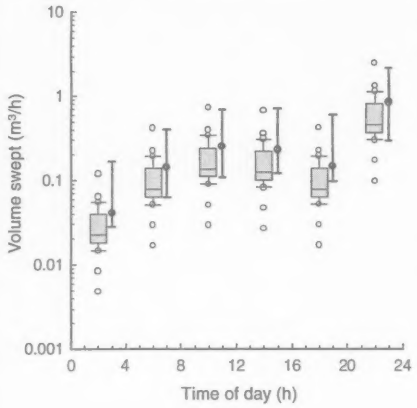

Figure 4

Estimated effective volume swept (K), with ±1 standard error (solid symbols and thick error bars) based on mean number of eggs per stomach and mean density of eggs from plankton collections, in relation to time of day (offset by +1 hour from midpoint of interval). Confidence intervals are based on the maximum likelihood estimates of mean numbers of eggs per capelin stomach. Box plots show the 25th, 50th, and 75th percentiles of the estimated effective volume swept (K) based on the mean number of eggs per stomach and each estimate of egg density based on plankton collections; whiskers show the 10th and 90th percentiles of the distribution and open symbols show the outlying points.

in estimated K using each observation of egg density from the plankton samples. This calculation generally resulted in less variability in the estimate of effective volume swept than the uncertainty in eggs per stomach (Fig. 4, box plots) because of the skewed distribution of egg densities based on plankton tows. The latter reflects the skewed distribution of egg densities based on plankton tows.

Discussion

Capelin prey composition observed in this study is consistent with that observed in previous studies, which show that copepods, euphausiids, and amphipods represent the principal prey items; the overall results of this study may therefore have general applicability. Fish eggs represent a relatively minor part of the diet of capelin. Neither Huse and Toresen (1996) nor Assthorsson and Gislason (1997) reported any occurrence of pelagic fish eggs in the stomachs of capelin from the Barents Sea and waters north of Iceland. O'Driscoll et al. (2001) reported an occurrence of less than 1% in capelin sampled during May–June in inshore waters and during August–September in offshore areas off Newfoundland. In the present study, an occurrence of 4% to 8%, which contributed an average of 1% of prey by numbers, was found for collections in coastal Newfoundland waters during July. There is little basis for explaining the differences between studies because only this analysis provides an estimate of the density of eggs in the water column where capelin were sampled. Fish eggs were approximately 1% of the prey found in the stomachs of capelin but they represented slightly more than 0.1% of the organisms sampled by the plankton nets used in this study. Huse and Toresen (1996) collected plankton data with different gear than that used in the present study, but they did not report the occurrence of pelagic fish eggs in their samples, possibly because pelagic fish eggs were in very low abundance or were absent during the collection periods of their study.

In general, fish eggs were more likely to be found in capelin stomachs as the length of individual fish increased. These fish tend to feed more heavily on larger prey types (e.g., euphausiids and amphipods) but the occurrence of fish eggs in individual fish tended to increase as the relative stomach fullness decreased. When considered with the general pattern in feeding periodicity, fish eggs were more likely to be eaten at the onset of feeding activity shortly after dusk, but as individual fish filled their guts with larger or more numerous prey, feeding on fish eggs tended to decrease. Fish eggs may represent an attractive prey for capelin capable of feeding on larger prey types (e.g., stage V of *C. finmarchicus*, euphausiids, and amphipods) because their weight is comparable to that of large copepods (Darbyson et al., 2003). However, as larger prey types are encountered or as hunger decreases, capelin may become more selective feeders and focus their foraging on energetically more profitable prey. The diurnal pattern of occurrence of fish eggs in capelin stomachs may also reflect differences in the visibility of eggs as a result of changing light levels, as well as predator hunger. Alternatively, the greater number of eggs in stomachs at dusk may reflect increased spatial overlap between predator and prey as capelin move toward the surface in the evening to feed (O'Driscoll et al.[4]).

The effective volume swept by capelin estimated in the present study indicates that the probability that a capelin will attempt to eat a pelagic fish egg is low, because it is highly unlikely that fish will swim at a very slow speed based on an assumed reactive distance. The predation rates based on average densities and encounter rates range from 0.04 to 0.86 egg/h

[4] O'Driscoll R. L., J. T. Anderson, and F. K. Mowbray. 2001b. Abundance and distribution of capelin from an acoustic survey in conjunction with the 1999 pelagic juvenile survey. Capelin in SA2 + Div. 3KL during 1999, Canadian Science Advisory Secretariat Research Document 2001/161. [Available from http://www.dfo-mpo.gc.ca/csas/ or Canadian Science Advisory Secretariat, Department of Fisheries and Oceans, 200 Kent Street, stn. 12032, Ottawa, Ontario, Canada K1A 0E6.]

based on an evacuation rate of 50% of eggs per hour. A low predation rate on fish eggs is not entirely unexpected. The eggs of cunner and cod from our samples had an average diameter of 0.86 and 1.3 mm, whereas the modal length of capelin from our collections was approximately 130 mm, making the eggs ~1% of the predator's body length. Based on Paradis et al.'s (1996) analysis, maximum predation rates by fish feeding on ichthyoplankton occur when the prey are 10% of the predator's length, and the minimum predation rates were observed when prey were ~0.5–1% of the predator's length. Paradis et al.'s (1996) summary equations indicate that an average predation rate of 0.007–0.03 eggs/h would not be inconsistent with laboratory estimates of predation rates for a range of evacuation times from 1 to 4 hours and would be considerably lower than the predation rates estimated in the present study. However, estimates based on a simple foraging model (e.g., Paradis et al., 1999) for a modal capelin swimming at 1 body length/s and with a reactive distance of 1 body length yield a mean of 12 eggs/h, and halving these parameter values yields a predation rate of 1.6 eggs/h. These alternative methods of estimating predation rates indicate that capelin are somewhat more likely to feed on fish eggs in the field than laboratory studies would indicate but less likely than would be anticipated from a simple foraging model. The results from this study do not provide an exhaustive estimate of encounter rates between capelin and pelagic fish eggs, but the range of effective encounter rates derived from the different approaches highlights the lack of understanding of this key parameter essential for any model of multispecies interactions. Such a broad range of expected predation (and encounter) rates indicates that in order to develop predictive skills in estimating the potential impact of planktivores on fish eggs, a better understanding of the factors that prompt a predator to feed on a fish egg is needed. Most laboratory studies dealing with predation by fish on fish eggs and larvae have used single prey and few have provided a measure of the probability of attack (Bailey and Houde, 1989; Paradis et al., 1996). Feeding patterns can be affected by the complexity of the available prey community (Kean-Howie et al., 1988; Gotceitas and Brown, 1993; Pepin and Shears, 1995), but methods to relate prey consumption with prey availability in the field are still limited.

Uncertainty in encounter and predation rates are due partly to errors in the estimation of prey availability and stomach content and partly due to our general lack of knowledge concerning the evacuation rate of fish eggs from predator stomachs. For this study I relied on Hunter and Kimbrell's (1980) observations of evacuation rates for northern anchovy from experiments conducted at 15°C. Water temperatures in Trinity Bay in July 2000 showed considerable variation with depth, ranging from ~13°C at the surface to –1°C at 100 m, with the overall average temperature over the water column being ~3–4°C. Given that most metabolic processes increase by 1.3–2 times over ten degrees Celsius (e.g., Brett and Groves, 1979), evacuation rates in the present study could have been half of those measured by Hunter and Kimbrell (1980) and thus would imply that our observations of stomach contents could represent 70% of the eggs consumed in the last hour rather than the 50% assumed in the analysis. The result would be that estimates of volumes swept by capelin would have to be reduced by ~30%, further reducing their potential impact on ichthyoplankton populations. The general uncertainty in our knowledge of evacuation rates for fish eggs from the stomachs of capelin, and from most other planktivorous fish, therefore limits the inferences that can be derived about their impact on ichthyoplankton survival.

A second source of uncertainty is due to the inherent sampling variability associated with the study of elements that form a small fraction of the prey of a predator. This study was based on more than 1000 specimens from a small region in order to provide the greatest accuracy possible within the confines of the study area. Stomach sampling is often restricted to fewer specimens sampled over a much broader geographic range. Without a large number of observations from a local environmental setting (i.e., site), estimates of mean numbers of rare prey types would likely be unable to reflect the effect of site-specific differences in environmental conditions on predator feeding and would thereby increase the uncertainty around the estimated effective encounter rates. The situation is well illustrated by the doubling in the frequency of occurrence of fish eggs in the specimens that were randomly selected for complete gut analysis versus all the specimens collected for analysis. Further uncertainty comes from the variability in estimates of egg density encountered by capelin—a variability that tended to result in slightly tighter confidence intervals in estimates of effective volume swept than the uncertainty due to variability in the number of prey per predator stomach. Variations in egg densities from plankton samples did result in lower estimates of effective volume swept, partly as a result of the rare high densities (typical of highly skewed distribution) that characterize the variability in plankton catches (Power and Moser, 1999). Although this study is not intended to provide a general estimate of the effective encounter rate of capelin feeding on fish eggs, the approach provides a useful example of the sampling requirements for the study of feeding on prey that are a minor part of the diet of a predator but which may be greatly affected by the predator's impact on the prey population.

Greater efforts must be directed at understanding patterns of predation in the field, in relation to prey availability, if the role of planktivorous fish in ichthyoplankton dynamics is to be understood. The assumptions in the estimation approach could affect the effective volume swept: estimates of the effective volume swept are based on average prey densities integrated over the water column, and the simplification of the encounter model (Eq. 1) incorporates possible variations in the probability of attack (e.g., due to diurnal variations in visibility of eggs) into the value of K. Pepin et

al. (2005) noted that in the study region, pelagic fish eggs are generally more abundant near the surface and decrease exponentially in abundance with increasing depth. As a result, if feeding by capelin occurs predominantly in surface waters, the effective density of prey encountered would be greater than the average density over the water column, which would in turn decrease the estimate of the effective volume swept based on our estimates of encounter rates. However, the patterns of vertical distribution of both eggs (Pepin et al., 2005) and capelin (O'Driscoll et al., 2001) are highly variable among sites and without accurate estimates of these elements from the current study, inferences about the impact on estimates of the effective volume swept would be speculative. In contrast, I implicitly incorporated the probability of attack into the estimate of the effective volume search, essentially giving it a value of 100%. However, Wieland and Köster (1996) found that the visibility of eggs increased with developmental stage and hence may alter the probability of attack by a predator. Lower visibility of early stage eggs could lead to an increase in the estimate of the effective volume swept, but because we could not stage most eggs sampled from the stomachs, the net effect on the estimated volume swept is unclear.

Planktivorous fish represent important forage for piscivores in several marine ecosystems but they can also play a significant role as predators of early life stages. In the Baltic Sea, spratt (*Sprattus sprattus* L.) feed extensively on cod eggs (Köster and Möllmann, 2000) and they may play an important role in regulating the underlying form of the stock-recruitment relationship (Köster et al., 2001). In upwelling systems, anchovies and sardines may also have a significant impact on egg and larval mortality rates (Smith et al., 1989). It has also been suggested that herring (*C. harengus* L.) and mackerel (*Scomber scombrus* L.) predation on ichtyplankton may play an important role in fish population dynamics in the Gulf of St. Lawrence (Swain and Sinclair, 2000) and on Georges Bank (Garrison et al., 2002). However it is unclear whether capelin play a significant role in the fish community dynamics in the ecosystems they inhabit, despite evidence that they are likely to have a significant impact on zooplankton abundance (Vésin et al., 1981; Akenhead et al., 1982; Skjoldal and Rey, 1989; Hassel et al., 1991). Clupeids, scombrids and engraulids can alternate between filter- and particulate-feeding modes, which may allow them to more effectively consume relatively small prey (~1 mm), such as fish eggs. There is no information on the feeding modes of capelin, but another osmerid, rainbow smelt (*Osmerus mordax*, Mitchill 1814), is known to be a primarily particulate feeder (Mills et al., 1995). Particulate feeding is generally directed toward larger prey, whereas filter feeding is used when prey are smaller or more abundant. Gill raker structure does not provide significant insight into the possible feeding modes of these various planktivorous fish. The estimate of reactive distance from the present study (0.024–0.034 m) is of the same order as the gape width of capelin (3.6% for TL; Pepin, unpubl. data), making filter feeding a distinctly possible mode of feeding. For known filter feeders, Uotani (1985) reported a maximum gill raker length of 6 mm in *Engraulis japonicus* (100 mm TL), Gibson (1988) reported an average gill raker length of 4 mm in *Clupea harengus* (150 mm TL), and Molina et al. (1996) reported gill raker lengths ranging from 5 to 9 mm in *Scomber japonicus* (150–250 mm SL). On the other hand, Lecomte and Dodson (2004) reported gill raker lengths of 3.3–3.9 mm in *Osmerus mordax* (150 mm TL). In the case of capelin, I found that gill rakers ranged from 3.0–4.5 mm in length (130–170 mm TL) (Pepin, unpubl. data). Inter-raker gaps among all these species ranges from 0.19 mm (*E. japonicus*) to 0.4 mm (*S. japonicus*) and from 0.36 to 0.45 mm in capelin (Pepin, unpubl. data). Thus all these species, which feed extensively on zooplankton but which use different ingestion modes, are equipped with gill rakers designed to retain small particles. To better understand the role of capelin in Arctic ecosystems, a greater knowledge of feeding will be required. If filter feeding is a dominant feeding mode, a simple filtration or volume-swept model may provide an accurate measure of the potential impact of capelin on fish eggs, whereas greater knowledge of behavior and selectivity will be required if particulate feeding is the dominant feeding mode because selection for or against fish eggs could be influenced by availability of alternate prey (e.g., Kean-Howie et al., 1988).

In most of the areas where planktivores can have a significant impact on the mortality of fish eggs, the ecosystems can be characterized as being "wasp-waisted" (Cury et al., 2000), i.e, as having a relatively high diversity of plankton species and top predators that are all influenced by the abundance and productivity of relatively few and dominant forage fish species that act to transfer energy from secondary producers to upper trophic levels in a manner similar to that found in many Arctic regions. It is clear that capelin are important prey for a number of top predators in the waters of the Barents Sea (Gjøsæter, 1998), Iceland (Vilhjálmsson, 2002), and on the Newfoundland and Labrador coast (Akenhead et al., 1982), and they may also have an important impact on the plankton community (Skjoldal and Rey, 1989; Hassel et al., 1991). What remains uncertain is whether they play an important regulatory role in the dynamics of the early life stages of fish. Observations from this study revealed that the highest occurrence of fish eggs in the stomachs of capelin came from capelin near one of the few remaining major spawning aggregations of the northern cod stock (Rose, 2003). The dynamics of such circular prey-predator interactions need to be studied further in order to understand their potential importance to population regulation (Walters and Kitchell, 2001) but to do so will require consideration of the spatial and temporal patterns of predator-prey interactions if predictive relationships are to be derived (Pepin et al., 2002, 2003; Baumann et al., 2003).

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A production modeling approach to the assessment of the horseshoe crab (*Limulus polyphemus*) population in Delaware Bay

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Abstract—Horseshoe crab (*Limulus polyphemus*) is harvested commercially, used by the biomedical industry, and provides food for migrating shorebirds, particularly in Delaware Bay. Recently, decreasing crab population trends in this region have raised concerns that the stock may be insufficient to fulfill the needs of these diverse user groups. To assess the Delaware Bay horseshoe crab population, we used surplus production models (programmed in ASPIC), which incorporated data from fishery-independent surveys, fishery-dependent catch-per-unit-of-effort data, and regional harvest. Results showed a depleted population (B_{2003}/B_{MSY}=0.03–0.71) and high relative fishing mortality (F_{2002}/F_{MSY}=0.9–9.5). Future harvest strategies for a 15-year period were evaluated by using population projections with ASPICP software. Under 2003 harvest levels (1356 t), population recovery to B_{MSY} would take at least four years, and four of the seven models predicted that the population would not reach B_{MSY} within the 15-year period. Production models for horseshoe crab assessment provided management benchmarks for a species with limited data and no prior stock assessment.

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The horseshoe crab (*Limulus polyphemus*) has become a source of controversy on the Atlantic coast of the United States (Berkson and Shuster, 1999; Walls et al., 2002). This species is commercially harvested for use as bait, is used by the biomedical industry, and is an important source of food for a large number of species, including migrating shorebirds. However, population trends in the Delaware Bay region in recent years have indicated a possible decline in horseshoe crab abundance, raising concerns that the population may be unable to fulfill the present and future needs of these diverse user groups.

Horseshoe crabs are harvested commercially for use as bait in the American eel (*Anguilla rostrata*), whelk, and conch (family Melongenidae) pot fisheries (ASMFC[1]). Historically, horseshoe crabs were considered "trash fish" of little commercial value and were used primarily as fertilizer or animal feed. When the bait fishery began, there were few restrictions on harvest and no harvest-reporting requirements. A maximum reported coastwide harvest of about 2 million crabs (3100 metric tons [t]) occurred in 1998 (ASMFC[2]). Commercial harvest has decreased in recent years owing to the adoption of state-by-state quotas in 2000 (ASMFC[3]) and the increased use of bait-saving devices for the eel and conch fisheries, both of which have reduced the demand for crabs.

Horseshoe crabs are also used by the biomedical industry. The blood of horseshoe crabs contains *Limulus* Amebocyte Lysate (LAL), a substance used to detect the presence of endotoxin contamination in injectable and implantable drugs and devices (Novitsky, 1984). The Food and Drug Administration estimated that 260,000 horseshoe crabs were bled for LAL in 1997. After bleeding, the animals were released at the capture site, and

[1] ASMFC (Atlantic States Marine Fisheries Commission). 1998. Interstate fishery management plan for horseshoe crab, 57 p. ASMFC, 1444 Eye Street, NW, Sixth Floor, Washington, DC 20005.

[2] ASMFC. 2004. Horseshoe crab 2004 stock assessment report, 87 p. ASMFC, 1444 Eye Street, NW, Sixth Floor, Washington, DC 20005.

[3] ASMFC. 2000. Addendum I to the fishery management plan for horseshoe crab, 9 p. ASMFC, 1444 Eye Street, NW, Sixth Floor, Washington, DC 20005.

the mortality from the bleeding process was estimated to be 7.5% (Walls and Berkson, 2003). Currently, there is no substitute for LAL that offers comparable speed and sensitivity.

Horseshoe crabs also play an important role in marine and terrestrial food webs (Botton and Shuster, 2003). Shorebirds migrating from South America to Arctic breeding grounds stop in the Delaware Bay to rebuild depleted energy reserves (Botton et al., 1994). The time and place of their stop-over coincides with that of annual horseshoe crab breeding, when the crabs arrive en masse to spawn on sandy beaches during high tides of May and June (Botton and Harrington, 2003). An adult female horseshoe crab lays approximately 88,000 eggs per year (Shuster, 1982), and a single red knot (*Calidris canutus*) can consume an estimated 18,000 crab eggs daily (USFWS[4]).

Horseshoe crabs account for substantial economic value in the Delaware Bay region. Regional economic contribution of the eel and conch fisheries is approximately $2.2 to $2.8 million annually. The regional economic value of the horseshoe crab biomedical industry is $26.7 to $34.9 million annually. Ecotourism related to migrating shorebirds has become increasingly important to the economy of the Delaware Bay region. An estimated 6,000 to 10,000 recreational bird watchers visit the Delaware Bay in spring and contribute $6.8 to $10.3 million to the regional economy.

Recently, there has been concern that the horseshoe crab population can fulfill the current needs of these user groups. These concerns led the Atlantic States Marine Fisheries Commission (ASMFC) to develop a fishery management plan for horseshoe crabs. Unfortunately, very few abundance data are available for this species. Many state and federal trawl surveys record horseshoe crabs caught during sampling, but gear and sampling methods are not designed for horseshoe crabs and catches are not common. Only recently have statistically robust horseshoe crab-specific surveys been initiated: a Delaware Bay spawning survey (Smith et al., 2002) and an offshore trawl survey (Hata and Berkson, 2003).

Because of the limited data available, previous stock assessments of the Delaware Bay population have been restricted to trend analyses to determine whether a single survey identifies a significant change in the population or whether there is a consensus among data sets. However, many Delaware Bay surveys have high variability and low power to detect population change and would therefore only be able to identify dramatic changes in population size. Also, trend analyses do not provide estimates of stock status (Caddy, 1998) such as relative biomass (B/B_{MSY}) and relative fishing mortality (F/F_{MSY}). The ultimate goal for horseshoe crab assessment employs a stage-based catch-survey methodology (Collie and Sissenwine, 1983; HSC-SAS[5]), incorporating data from harvest and surveys. It will be a number of years before this modeling approach can be implemented, however, since stage-class data from commercial harvest are not currently being collected.

The surplus production modeling approach (Prager, 1994) used in our study is an appropriate bridge between these two methods. Production models allow for the incorporation of harvest data and multiple surveys, improving predictive power over that of a single survey. This technique does not include a stage-structure in the model; instead it focuses on the dynamics of the population as a whole. Similar methods have been successfully applied to horseshoe crab data from Rhode Island (Gibson and Olszewski[6]) and production models have been widely used for assessments of other species (Booth and Punt, 1998; Cadrin and Hatfield, 2002; Vaughan and Prager, 2002). Surplus production models assume a low population growth rate at small population sizes and as the population nears the carrying capacity (Quinn and Deriso, 1999). In the logistic growth form of the model used in the present study, maximum growth rate (and maximum surplus production) occurs at one-half of the carrying capacity. At this point, the maximum surplus population growth can be harvested while still maintaining a stable population size. Surplus production models provide estimates of maximum sustainable yield (MSY; the largest harvest that can continuously be removed from a stock), population biomass, and fishing mortality, as well as allow for the estimation of effects of future management.

We fitted a regional-scale production model to Delaware Bay horseshoe crab data in order to quantify the current status of the Delaware Bay population and to estimate impacts of future management actions. The results from this production model will allow the ASMFC and member states to manage the Delaware Bay population of horseshoe crabs more effectively with the goal of providing a sustainable resource for commercial harvest, the biomedical industry, and migrating shorebirds.

Methods

Production model

We used an age-aggregated production model with the Prager (1994) form of the Graham-Schaefer surplus-production model (i.e., logistic population growth),

[4] USFWS (U.S. Fish and Wildlife Service). 2003. Delaware Bay shorebird-horseshoe crab assessment report and peer review. Migratory Bird Publication R9-03/02, 107 p. USFWS, 4401 N. Fairfax Dr., MBSP 4107, Arlington, VA 22203.

[5] HSC-SAS (Horseshoe crab stock assessment subcommittee). 2000. A conceptual framework for the assessment of horseshoe crab stocks in the mid-Atlantic region, 19 p. ASMFC, 1444 Eye Street, NW, Sixth Floor, Washington, DC 20005.

[6] Gibson, M., and S. Olszewski. 2001. Stock status of horseshoe crabs in Rhode Island in 2000 with recommendations for management, 13 p. RI Division of Fish and Wildlife, 4808 Tower Hill Rd., Wakefield, RI 02879.

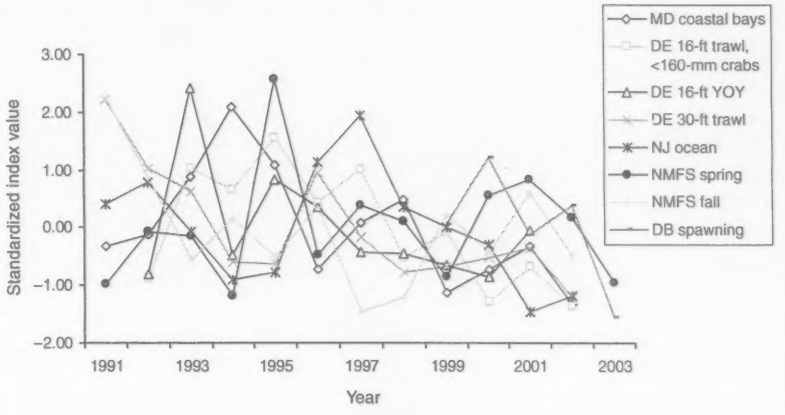

Figure 1

Fishery-independent survey indices for the Delaware Bay (DB) region from 1991 through 2003, standardized by survey for comparison. MD = Maryland; DE = Delaware; NJ = New Jersey; DB=Delaware Bay.

$$\frac{dB_t}{d_t} = (r - F_t)B_t - \frac{rB_t^2}{K},$$

where r = the stock's intrinsic growth rate;
K = the carrying capacity, both of which are assumed to be constant (Prager, 1994); and
F_t and B_t = Fishing mortality and biomass, respectively, at time t.

In addition, the harvest was not assumed to equal surplus production (i.e., the model was a dynamic or non-equilibrium model; Quinn and Deriso, 1999). This model assumes $B_{MSY} = 0.5K$, where B_{MSY} is the spawning biomass that would produce MSY. This form is often used because of its theoretical simplicity and because it is central among possible production model shapes. This production model was conditioned on catch, meaning that landings data were assumed to be more precise than abundance indices. By assuming that abundance indices are correlated measures of population abundance, the model is able to incorporate multiple indices by interpreting differences among indices as sampling error. To fit the production model, we used the ASPIC software (vers. 5.02) of Prager (1994), a program that has been used extensively in stock assessments (Cadrin and Hatfield, 2002; MacCall, 2002). We included data from fishery-independent and fishery-dependent sources in model runs.

Abundance indices

In the Delaware Bay region, there are a number of fishery-independent surveys that collect data on horseshoe crabs (Fig. 1). These include National Marine Fisheries Service (NMFS) trawl (spring, 1968–2003, and fall, 1963–2002), New Jersey (NJ) ocean trawl (1989–2002), Maryland (MD) coastal bays trawl (1988–2002), Delaware (DE) 16-ft trawl (juvenile and young-of-the-year; 1992–2002), DE 30-ft trawl (1990–2002), and Delaware Bay spawning survey (1999–2003). Detailed descriptions of these surveys can be found in ASMFC.[2] We selected 1991–2003 as the modeling timeframe because both harvest data and abundance index data were available for this period.

In spite of the large number of surveys and long time series for some of these surveys, many had high variability and low power to detect a decline. Additionally, many of these surveys were negatively correlated with each other for the years investigated (Table 1). Because an underlying assumption for the model is that each survey is representative of the population being evaluated, total disagreement (i.e., negative correlation) among any pair of surveys cannot be reconciled by the model, resulting in model errors. We therefore used three subsets of fishery-independent surveys in which all pairs were positively correlated for population modeling (Table 2), incorporating six of the eight fishery-independent surveys into production model runs. Fishery-dependent data were also available from 1991 to 2002 from the Delaware hand and dredge fisheries. Abundance indices based on catch-per-unit-of-effort (CPUE) for these fisheries were calculated from the annual number of trips and landings for each fishery (Fig. 2).

Within the models, abundance indices were weighted by the inverse of the coefficient of variation (CV) from regressions, which gave more weight to surveys with less variability. For comparison, we also conducted model

runs where surveys were weighted equally within the models. Table 2 lists the three fishery-independent models (referred to as FI) and the four fishery-dependent models (referred to as FD) used in production model applications and the CV and weighting of each survey within the models.

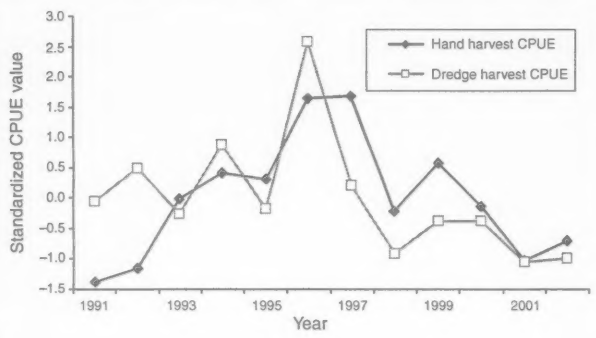

Figure 2

Fishery-dependent abundance indices based on catch-per-unit-of-effort data for the Delaware hand and dredge horseshoe crab (*Limulus polyphemus*) fisheries, 1991–2002, standardized by index for comparison.

Harvest

We obtained horseshoe crab harvest data for Virginia, Maryland, Delaware, Pennsylvania, and New Jersey from 1995 to 2003 (NMFS[7]; Michels[8]). We combined harvests among states for regional-scale production model runs. We estimated the regional harvest from 1991 to 1994 from available Delaware landings data (Fig. 3). Reporting of harvest was not mandatory during this time period, and harvest data

[7] NMFS (National Marine Fisheries Service). 2004. Fisheries Statistics and Economics Division, Silver Spring, MD. Landings data (by state and year) obtained in August 2004 from http://www.st.nmfs.gov/st1/commercial/index.html. Web page was last modified 24 September 2003.

[8] Michels, S. Personal commun. 2004. DE Div. of Fish and Wildlife, 89 Kings Highway, Dover, DE 19901.

Table 1

Correlation matrix for abundance indices derived from 10 surveys, with number of pairwise comparisons (i.e., years) in parentheses for 1991–2003. Negatively correlated indices are shown in bold font. Indices used in production model runs are identified with an asterisk (*).

	1 NMFS fall	2 NMFS spring	3 DE 30-ft trawl	4 DE 16-ft trawl, <160-mm crabs	5 DE 16-ft trawl, YOY	6 NJ ocean	7 MD coast	8 Spawning survey	9 DE dredge	10 DE hand
1 NMFS sall	1.000 (12)									
2 NMFS spring*	**−0.409** (12)	1.000 (13)								
3 DE 30-ft* trawl	0.706 (12)	**−0.370** (12)	1.000 (12)							
4 DE 16-ft trawl, <160-mm* crabs	**−0.261** (11)	0.182 (11)	0.215 (11)	1.000 (11)						
5 DE 16-ft trawl, YOY*	**−0.153** (10)	0.214 (10)	0.341 (10)	0.617 (10)	1.000 (10)					
6 NJ ocean*	**−0.102** (12)	**−0.249** (12)	0.479 (12)	0.252 (11)	**−0.132** (10)	1.000 (12)				
7 MD coastal bays*	**−0.181** (12)	0.086 (12)	**−0.038** (12)	0.639 (11)	0.336 (10)	**−0.116** (12)	1.000 (12)			
8 Spawning survey	**−0.786** (4)	0.211 (5)	**−0.069** (4)	**−0.657** (4)	**−0.781** (3)	0.392 (4)	**−0.087** (4)	1.000 (5)		
9 DE dredge harvest*	0.210 (12)	**−0.329** (12)	0.450 (12)	0.379 (11)	0.031 (10)	0.506 (12)	0.135 (12)	0.509 (4)	1.000 (12)	
10 DE hand harvest*	**−0.536** (12)	0.002 (12)	**−0.214** (12)	0.639 (11)	0.084 (10)	0.485 (12)	0.123 (12)	0.167 (4)	0.564 (12)	1.000 (12)

from other states were unavailable or unreliable. We therefore expanded the Delaware harvest to represent the Delaware Bay region by making the assumption that regional landings were equal to ten times the landings in Delaware, the approximate relationship from 1997 through 2003 when reporting was mandatory. When applicable, we converted numbers to metric tons (t) (Prager and Goodyear, 2001) using the relationship of Gibson and Olszewski[6] (1.8182 kg/horseshoe crab). Commercial harvest of horseshoe crabs has been below the regional quota of 2595 t since 2000 (Fig. 3).

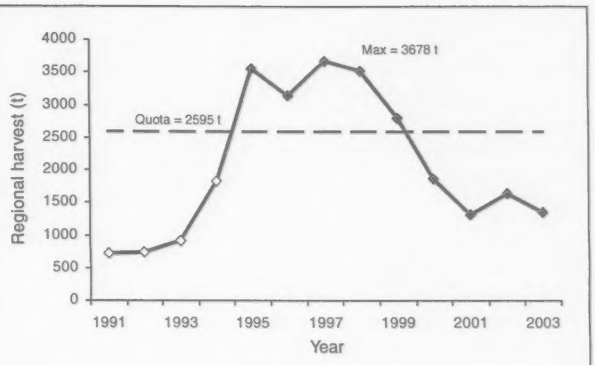

Figure 3

Delaware Bay regional horseshoe crab (*Limulus polyphemus*) harvest (in t). Regional harvest, 1991–1994 (open diamonds), was estimated to be ten times the Delaware harvest. The current regional quota of 2595 t is shown by the horizontal line.

Assumptions associated with the production models

There were a number of general assumptions associated with production models (Quinn and Deriso, 1999). We assumed that productivity (change in biomass over time) responded instantaneously to changes in population size. Changes in the biotic and abiotic environments were ignored, and r (the intrinsic rate of population growth) and K (the carrying capacity) were assumed to be constant. Because production models combine all age classes, it was assumed that size or age structure of the population would not have major effects on population dynamics.

Specific assumptions about population values were also required by the model. All starting values of MSY and K were based on the maximum harvest in the Delaware Bay from 1991 through 2003. The initial guess for MSY was 1850 t (half of the largest catch), and the initial guess for K was 37,000 t (ten times the largest catch). For fishery-independent model runs, we had the model freely estimate initial biomass in relation to carrying capacity (B_1/K), with our start-

Table 2

Description of production model runs, including the data sources, years, and coefficient of variation (CV) from regression analyses. The weighting of surveys within the models is the inverse of the CV. The starting estimate of B_1/K for each model is also shown. FI refers to models that include fishery-independent abundance indices, and FD identifies models where fishery-dependent indices from CPUE data were used.

Model	Data sources	Years	CV	Relative weighting	Initial B_1/K value
FI-1	NMFS spring	1991–2003	0.636	0.241	0.5
	DE 16-ft trawl, <160-mm crabs	1992–2002	0.517	0.296	
	DE 16-ft trawl YOY	1992–2001	0.928	0.165	
	MD coastal bays	1991–2002	0.515	0.297	
FI-2	DE 30-ft trawl	1991–2002	0.524	0.388	0.5
	DE 16-ft trawl, <160-mm crabs	1992–2002	0.517	0.393	
	DE 16-ft YOY	1992–2001	0.928	0.219	
FI-3	DE 30-ft	1991–2002	0.524	0.283	0.5
	DE 16-ft trawl, <160-mm crabs	1992–2002	0.517	0.287	
	NJ ocean trawl	1991–2002	0.345	0.430	
FD-1	DE hand CPUE	1991–2002	0.449	0.553	Fixed at 0.1
	DE dredge CPUE	1991–2002	0.555	0.447	
FD-2	DE hand CPUE	1991–2002	0.449	0.553	Fixed at 0.2
	DE dredge CPUE	1991–2002	0.555	0.447	
FD-3	DE hand CPUE	1991–2002	0.449	0.553	Fixed at 0.3
	DE dredge CPUE	1991–2002	0.555	0.447	
FD-4	DE hand CPUE	1991–2002	0.449	0.553	Fixed at 0.4
	DE dredge CPUE	1991–2002	0.555	0.447	

ing estimate of this value equal to 0.5. In the absence of other information about starting biomass, B_1/K = 0.5 (i.e., B_1=B_{MSY}) is an appropriate default value (Punt, 1990; Prager, 1994). In some cases B_1/K was poorly estimated, and we employed a common solution of fixing the value of B_1/K (Vaughan and Prager, 2002). For model runs based on fishery-dependent indices, we fixed B_1/K at 0.1, 0.2, 0.3, and 0.4 (Table 2). The CPUE value for the Delaware hand fishery was relatively low in 1991; therefore we assumed that the population biomass was below B_{MSY}, i.e. B_1/K was less than 0.5.

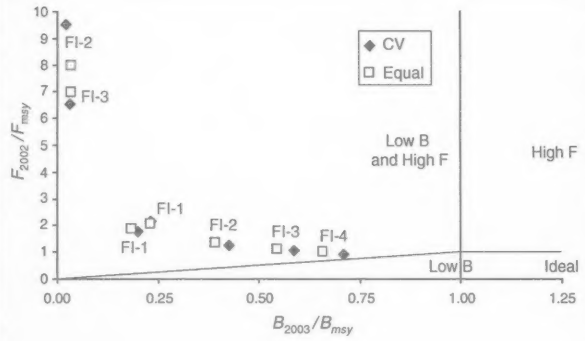

Figure 4

Relative 2003 biomass (B_{2003}/B_{MSY}) and relative 2002 fishing mortality (F_{2002}/F_{MSY}) of Delaware Bay horseshoe crab (*Limulus polyphemus*) for each of the seven model runs presented, with abundance indices weighted equally or inversely to CV within the models.

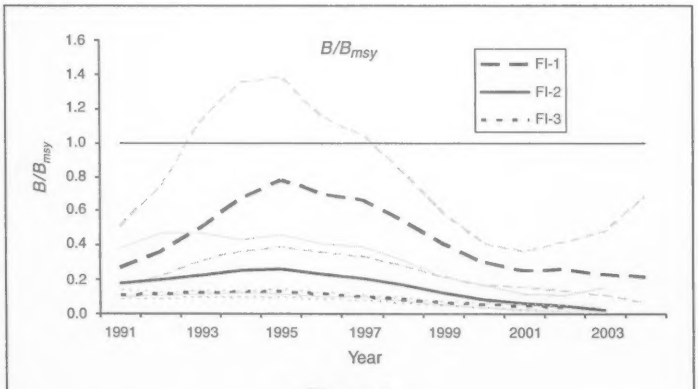

Figure 5

Production model estimates of relative biomass (B/B_{MSY}) of horseshoe crabs (*Limulus polyphemus*) in the Delaware Bay region, 1991–2004. Results from fishery-independent model runs are shown, and the horizontal line represents B/B_{MSY}=1. 80% confidence intervals each model run are the same line pattern in gray.

Quantities estimated by the model

The production model estimated several benchmarks and status indicators useful in understanding horseshoe crab biology and in improving management. These quantities included relative biomass (B/B_{MSY}), relative fishing mortality (F/F_{MSY}), population biomass (B), and maximum sustainable yield (MSY). Point estimates and 80% confidence intervals for each of these quantities were calculated for each model run.

Population projections

We used the Delaware Bay population estimates calculated by the surplus production model to project the population forward in time for a period of 15 years to evaluate potential harvest levels. We conducted projections using ASPICP (Prager, 1994), with annual landings specified for each year of the projections. We selected a 15-year time period because this is the longest projection period that can be programed in ASPICP, and confidence in projection model results decreases at longer time intervals. We evaluated trends in biomass over time for a range of harvest levels, including harvest at 2003 levels and proportional reductions of that harvest. These harvests were 0% of 2003 catch (no harvest), 25% (339 t annually), 50% (678 t), 75% (1017 t), and 100% (1356 t). We identified the number of years under each harvest scenario required for the population (and 80% confidence intervals) to rebuild to B_{MSY}. We also compared relative biomass (with 80% confidence intervals) in the final year of projections (2018) for each harvest level.

Results

Results differed little between model simulations for surveys weighted inversely to CV and for simulations with equally weighted surveys (Fig. 4).

B/B_{MSY}

Production model runs showed that B/B_{MSY} in the Delaware Bay region increased in the early 1990s and has declined steadily since 1995 (Figs. 5 and 6). Slight increases since 2001 were evident in some model runs. Relative biomass in 2003 was estimated to be low, with point estimates ranging from 0.03 to 0.20 for models with fishery-independent data and 0.20 to 0.71 for models with fishery-dependent data (Table 3). Eighty-percent confidence intervals for B_{2003}/B_{MSY} ranged from 0.005 to 1.16.

Table 3

Status indicators and management benchmarks for horseshoe crabs in the Delaware Bay region, estimated from production model runs with fishery-independent (FI) or fishery-dependent (FD) indices weighted by the inverse of the CV. Point estimates and lower (L) and upper (U) 80% confidence intervals (CIs) are shown for each model run. The objective function is a measure of how well the model was able to fit the data using a lognormal error structure—a lower value representing a better fit.

	FI-1	FI-2	FI-3	FD-1	FD-2	FD-3	FD-4
B_{2003}/B_{MSY}	0.232	0.022	0.030	0.201	0.427	0.588	0.710
80% CI (L)	0.109	0.005	0.008	0.127	0.238	0.319	0.388
80% CI (U)	0.479	0.154	0.041	0.347	0.762	1.015	1.157
F_{2002}/F_{MSY}	2.156	9.501	6.560	1.795	1.269	1.034	0.911
80% CI (L)	1.442	6.005	3.999	1.258	0.770	0.588	0.513
80% CI (U)	3.299	18.320	21.300	2.592	2.069	2.112	2.184
B_{2003} (t)	2197	1084	4098	2579	3653	5035	6604
80% CI (L)	1319	524	2295	1423	1853	2955	4340
80% CI (U)	4603	4306	9379	4372	5012	7778	12,080
MSY (t)	3064	5082	7220	3768	2628	2354	2196
80% CI (L)	2550	2985	5340	3274	2566	1905	1372
80% CI (U)	4475	8713	9577	4499	2787	2505	2501
Objective function	16.05	12.40	9.57	2.62	2.93	3.39	3.85

F/F_{MSY}

Although harvest of horseshoe crabs has decreased in recent years (Fig. 3), fishing mortality remains high (Figs. 7 and 8). F_{2002}/F_{MSY} point estimates ranged from 2.3 to 9.5 for models with fishery-independent data and from 0.9 to 1.8 for models with fishery-dependent data (Table 3).

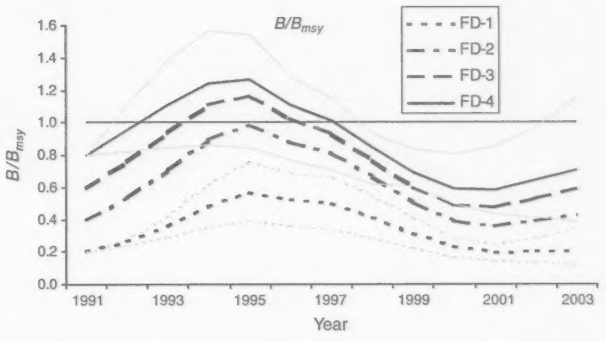

Figure 6

Production model estimates of relative biomass (B/B_{MSY}) of horseshoe crabs (*Limulus polyphemus*) in the Delaware Bay region, 1991–2003. Results from fishery-dependent model runs are shown, and the horizontal line represents B/B_{MSY}=1. 80% confidence intervals for models FD-1 and FD-4 are shown in gray.

Biomass

Biomass of horseshoe crabs in the Delaware Bay region has decreased substantially since 1995, such that the 2003 biomass was less than 56% of the 1995 biomass. This equates to an annual decline of greater than 7% during this period. Point estimates for 2003 biomass ranged from 1084 t (596,000 crabs) to 6604 t (3,632,000 crabs). As is characteristic of production models (Prager, 1994), absolute biomass was estimated much less precisely than relative biomass (B/B_{MSY}). The range of 80% confidence intervals for 2003 biomass across all seven model runs was 524 t (288,000 crabs) to 12,080 t (6,644,000 crabs).

Population projections

We used results from production model runs to project the horseshoe crab population forward in time to evaluate potential management options. Figure 9 shows the trajectory of B/B_{MSY} over time for model FI-1 under each harvest scenario. The number of years required to rebuild the population to B_{MSY} varied substantially among models (Table 4). At 2003 harvest levels (i.e., 100%), projections showed population recovery in a minimum of four years, although four of the seven models did not reach B_{MSY} in the 15-year projection period. In the absence of harvest (i.e., 0%), recovery could occur in as few as 2 years, but two models did not reach B_{MSY} in the projection period. Estimates of B/B_{MSY} in the final year of projections (2018) also differed among model applications (Table 5).

Discussion

According to surplus production model runs and corresponding projections, the horseshoe crab population in the Delaware Bay region has been depleted and current harvest levels may be too high to allow the population to rebuild to B_{MSY} within 15 years. Biomass in this region has decreased steadily since 1995 and is currently well below B_{MSY}. This decline was evident in models that incorporated regional fishery-independent surveys and Delaware fishery-dependent indices. Figure 4 shows current relative biomass and relative fishing mortality for each of the model applications. All model runs indicated a depleted population with high fishing mortality, although the estimated extent differed among models (Fig. 4). Projections for some of the model runs predicted a relatively fast population recovery. In the absence of harvest, five of the seven model applications predicted rebuilding to B_{MSY} in five or fewer years. However, two of the model runs estimated such low population biomass that rebuilding the Delaware Bay population to B_{MSY} would take greater than 15 years, even with no harvest. A precautionary management strategy may therefore be appropriate for this population in the short term as more data are being collected.

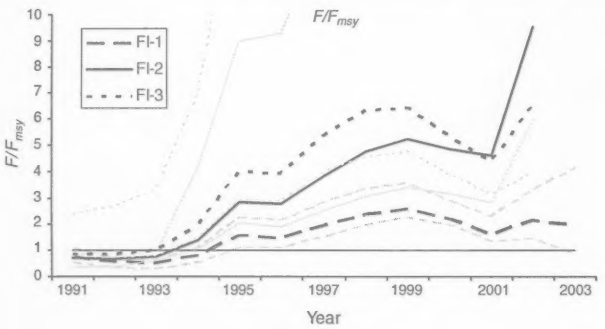

Figure 7

Production model estimates of relative fishing mortality (F/F_{MSY}) of horseshoe crabs (*Limulus polyphemus*) in the Delaware Bay region, 1991–2003. Results from fishery-independent model runs are shown, and the horizontal line represents F/F_{MSY}=1. 80% confidence intervals for each model run are the same line pattern in gray.

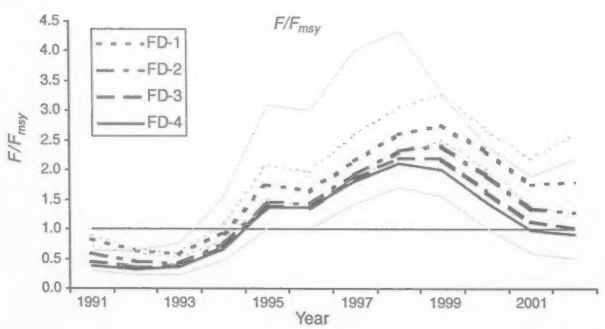

Figure 8

Production model estimates of relative fishing mortality (F/F_{MSY}) of horseshoe crabs (*Limulus polyphemus*) in the Delaware Bay region, 1991–2002. Results from fishery-dependent model runs are shown, and the horizontal line represents F/F_{MSY}=1. 80% confidence intervals for models FD-1 and FD-4 are shown in gray.

Population model results may have been affected by assumptions that we made during the modeling process. In order to extend the time period of the model back to 1991, we had to estimate regional harvest based on Delaware harvest data for the early years of the model. Because harvest data from most states were not available prior to 1995, we assumed that regional harvest was equal to ten times the Delaware harvest for the years 1991–94. This was the approximate relationship between Delaware and regional harvest from 1997 through 2003 when reporting was mandatory. Actual regional harvest for 1991–94 was unknown because there were no harvest-reporting requirements. We conducted sensitivity analyses which showed very little difference among runs with 1991–94 harvest equal to Delaware landings, 10 times Delaware landings, or 20 times the Delaware landings (the mean difference for B_{2003}/B_{MSY} from these runs was 0.011). If the actual harvest was substantially greater than 20 times Delaware landings, differences in results may occur.

Results were also influenced by the data sources that were included. We based the inclusion of fishery-independent surveys on positive correlations among surveys, incorporating the largest number of data sources possible into model runs. However, two fishery-independent surveys were not included: the NMFS fall trawl and the Delaware Bay spawning survey. The NMFS fall trawl was negatively correlated with seven of the nine other data sources (Table 1), and we therefore assumed that it was not a reliable index of horseshoe crab abundance. The spawning survey was negatively correlated with five of the nine other data sources (Table 1). This survey also had a very short time series because it was redesigned in 1999 to improve statistical power (Smith et al., 2002). With so few data points, the production model would be unable to distinguish population trends from survey variability; therefore spawning survey data were excluded. However, as one of only two horseshoe crab-specific surveys currently in place, the Delaware Bay spawn-

ing survey will likely prove to be a valuable source of information in the future.

Negative correlations were present among a relatively large number of Delaware Bay surveys (Table 1) which are assumed to be sampling the same population. This is a common problem in fisheries stock assessments (Richards, 1991; Schnute and Hilborn, 1993). The observed differences among these surveys could be attributed to a number of factors. Catches of horseshoe crabs are not common, leading to small sample sizes and high variability. Also, these surveys may differ in location, time of year, and gear selectivity. Future studies could employ an analysis of variance (ANOVA) to attempt to separate these factors from underlying horseshoe crab abundance trends.

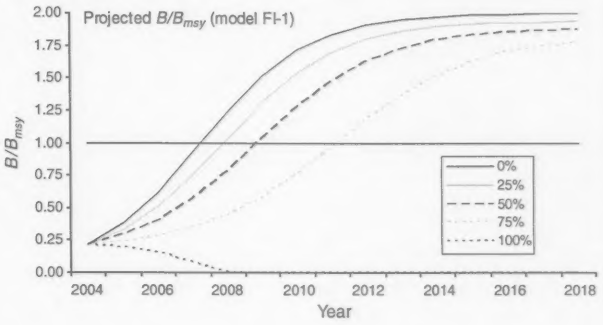

Figure 9

Example of projection results. Projected relative biomass (B/B_{MSY}) in shown for model FI-1, with each line representing a harvest level applied annually in the 15-year projections. The percentage refers to the percent of the 2003 Delaware Bay regional landings of 1356 t (i.e., 50%=678 t).

The data sources included in individual model runs also led to differing results. Models incorporating fishery-dependent data tended to present a slightly more optimistic view of the population and the fishery, predicting higher relative biomass and lower relative fishing mortality. Although harvest data often have the benefit of having been derived from large sample sizes (and have resulting low variance estimates), there is often a bias associated with fishery-dependent data. Fisheries do not sample randomly because they target areas of highest abundance, and thus biased indices are produced (Quinn and Deriso, 1999). In addition, the use of fishery-dependent abundance indices is often complicated by changes in gear, regulations, or sampling methods over time, any of which could affect catch rates. Fishery-independent surveys are usually more appropriate for assessments, assuming there is high consistency in sampling methods among years (Chen et al., 2003).

Differences also existed among fishery-independent surveys. Models FI-2 and FI-3 predicted a much lower population size than FI-1 or the fishery-dependent models. Projections with these runs predicted that the population was too depleted to recover in 15 years, even in the absence of harvest. The models differed only in the abundance indices included. In the trend analyses conducted for ASMFC,[2] the most significant population declines since 1997 were identified in the NJ ocean

Table 4

Results of Delaware Bay population projections from production model runs from fishery-independent (FI) and fishery-dependent (FD) indices. Projections were conducted for 15 years, with a constant harvest (in t) applied annually. Harvest levels were based on 2003 harvest and are listed in the left column. The time period (and 80% confidence intervals) shown represent the number of years (starting in 2003) required for the population biomass to reach B_{MSY}. "n/a" indicates that the biomass did not reach B_{MSY} during the 15-year projection period.

Harvest level in relation to that of 2003	Years to rebuild to B_{MSY}						
	FI-1	FI-2	FI-3	FD-1	FD-2	FD-3	FD-4
0% (0 t)	5 years	n/a	n/a	4 years	3 years	2 years	2 years
80% CI	(2, 12)	(3, n/a)	(n/a, n/a)	(3, 7)	(1, 6)	(0, 8)	(0, 15)
25% (339 t)	5	n/a	n/a	5	3	2	2
80% CI	(2, n/a)	(3, n/a)	(n/a, n/a)	(3, 8)	(1, 8)	(0, 9)	(0, n/a)
50% (678 t)	6	n/a	n/a	6	4	3	2
80% CI	(2, n/a)	(n/a, n/a)	(8, n/a)	(3, 11)	(1, 10)	(0, 14)	(0, n/a)
75% (1017 t)	9	n/a	n/a	7	4	3	3
80% CI	(3, n/a)	(n/a, n/a)	(5, n/a)	(4, 15)	(1, n/a)	(0, n/a)	(0, n/a)
100% (1356 t)	n/a	n/a	n/a	n/a	6	5	4
80% CI	(3, n/a)	(n/a, n/a)	(n/a, n/a)	(5, n/a)	(2, n/a)	(0, n/a)	(0, n/a)

Table 5

Results of Delaware Bay population projections from production model runs from fishery-independent (FI) and fishery-dependent (FD) indices. Projections were conducted for 15 years, with a constant harvest (in t) applied annually. Harvest levels were based on 2003 harvest and are listed in the left column. The relative biomass (B/B_{MSY}) in the final year of projections (2018) and 80% confidence intervals are shown for each model simulation.

Harvest level in relation to that of 2003	B_{2018}/B_{MSY}						
	FI-1	FI-2	FI-3	FD-1	FD-2	FD-3	FD-4
0% (0 t)	2.00	0.41	0.14	2.00	2.00	2.00	2.00
80% CI (L)	1.49	0.02	0.04	1.91	1.87	1.63	1.05
80% CI (U)	2.00	1.97	0.28	2.00	2.00	2.00	2.00
25% (339 t)	1.94	0.00	0.06	1.95	1.93	1.92	1.91
80% CI (L)	0.00	0.00	0.00	1.95	1.90	1.42	0.86
80% CI (U)	1.96	1.94	0.42	1.95	1.93	1.93	1.93
50% (678 t)	1.87	0.00	0.00	1.90	1.86	1.84	1.82
80% CI (L)	0.00	0.00	0.00	1.89	1.80	1.07	0.61
80% CI (U)	1.93	0.00	1.76	1.90	1.86	1.86	1.86
75% (1017 t)	1.78	0.00	0.00	1.83	1.78	1.74	1.71
80% CI (L)	0.00	0.00	0.00	1.66	0.86	0.57	0.26
80% CI (U)	1.85	0.00	1.74	1.85	1.78	1.78	1.77
100% (1356 t)	0.00	0.00	0.00	0.76	1.67	1.62	1.58
80% CI (L)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
80% CI (U)	1.72	0.00	0.00	1.77	1.69	1.69	1.68

trawl and the DE 16-ft 160-mm survey, both of which were included in model FI-3, and possibly explain the low population estimates.

In other trend analyses conducted during the previous assessment, a population decline in the Delaware Bay was less evident. Using data from 1997 through 2003, we found that only four of eight fishery-independent surveys showed a significant decline, partially owing to high variability and low power. By incorporating a number of these surveys into a production model, we also found that the decreasing biomass in recent years becomes more apparent. These production model runs provide the added benefit of estimating stock status and management benchmarks, as well as the benefit of evaluating future management options.

Although interpretation of absolute biomass or population size from a surplus production model can be somewhat problematic, our estimates are roughly comparable to estimates from previous studies. Hata and Berkson (2003) calculated a mean 2001 population size of 4.4 million adults (95% confidence intervals of 2.1 million and 6.8 million) in the Delaware Bay from daytime trawl survey data. Botton and Ropes (1987) estimated 2.3 to 4.5 million adults in this region. In the present study, our estimates of the 2003 population size ranged from 0.6 million to 3.6 million crabs, and the mean of the seven model runs equaled 2.0 million crabs. Eighty percent confidence intervals ranged from 0.3 million to 6.6 million crabs for all model applications. Although within the range of results from the other studies, these wide confidence intervals provide little information for management. It will therefore be more appropriate to interpret relative biomass (B/B_{MSY}) and relative fishing mortality (F/F_{MSY}) for use in management decisions (Prager, 1994).

It is important to understand the spatial scale and population represented by these models and analyses. This regional model is a compilation of a number of localized fishery-independent surveys, most of which encompassed a relatively small spatial area. However, the model results should not be interpreted at a more localized scale because landings data are combined for the region. Similarly, interpretation of results should not be expanded to represent other Atlantic horseshoe crab populations outside the Delaware Bay region because neither survey nor harvest data in this model extend to other regions. Nevertheless, the Delaware Bay is believed to be the center of abundance and spawning activity for Atlantic horseshoe crabs; therefore population trends in this region may have significant implications for adjacent populations.

In analyses conducted for ASMFC,[2] trend analyses identified dramatic regional differences in horseshoe crab population trends. Although the Delaware Bay, eastern Long Island Sound, and New England populations have experienced declines in recent years, the southeast population and the western Long Island population have remained stable or have increased. Future production models applied to these other regions will hopefully clarify these trends and allow managers to

determine regional harvest regulations. By identifying appropriate management for each region, we will improve our ability to rebuild the Atlantic horseshoe crab population and provide a sustainable resource for the diverse user groups.

Acknowledgments

This research was funded by the National Marine Fisheries Service. We thank M. Prager and M. Gibson for providing modeling advice, D. Smith, S. Michels, and B. Andres for providing horseshoe crab data and guidance, and the members of the Horseshoe Crab Stock Assessment and Technical Committees of the ASMFC for their contribution to this study. This manuscript was improved by the comments and suggestions from M. Prager, M. Millard, S. Michels, B. Murphy, D. Hata, and S. Klopfer, and two anonymous reviewers. We greatly appreciate the time and effort of all involved.

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Biodiversity as an index of regime shift in the eastern Bering Sea

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Abstract—Data collected from an annual groundfish survey of the eastern Bering Sea shelf from 1975 to 2002 were used to estimate biomass and biodiversity indexes for two fish guilds: flatfish and roundfish. Biomass estimates indicated that several species of flatfish (particularly rock sole, arrowtooth flounder, and flathead sole), several large sculpins (*Myoxocephalus* spp.), bigmouth (*Hemitripterus bolini*), and skates (*Bathyraja* spp.) had increased. Declining species included several flatfish species and many smaller roundfish species of sculpins, eelpouts (*Lycodes* spp.), and sablefish (*Anoplopoma fimbria*). Biodiversity indexes were calculated by using biomass estimates for both guilds from 1975 through 2002 within three physical domains on the eastern Bering Sea shelf. Biodiversity trends were found to be generally declining within the roundfish guild and generally increasing within the flatfish guild and varied between inner, middle, and outer shelf domains. The trends in biodiversity indexes from this study correlated strongly with the regime shift reported for the late 1970s and 1980s.

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Environmental and biological events in the North Pacific can indicate regime shifts or reorganizations of the ecosystem at the environmental and biological level (Hare and Mantua, 2000). Measurable climatic events were identified in the mid-1970s, late 1980s (Anderson and Piatt, 1999; Anderson, 2000; Hare and Mantua, 2000; Zhang et al., 2000; Minobe, 2002) and the late 1990s (Hunt and Stabeno, 2002; Minobe, 2002). Regime shifts in the eastern Bering Sea (EBS) have been correlated with several climatic events, including the Pacific Decadal Oscillation, the El Niño Southern Oscillation (Hollowed et al., 2001), sea ice coverage (Stabeno et al., 2001; Hunt et al., 2002; Hunt and Stabeno, 2002), and summer sea surface temperatures (Bond and Adams, 2002; Hunt et al., 2002; Minobe, 2002). The far reaching effects that climate change has on an ecosystem are not well defined, but many studies have shown strong correlations between climate change, fish recruitment, and plankton production in the North Pacific (Brodeur and Ware, 1992; Anderson et al., 1997; Anderson and Piatt, 1999; Brodeur et al., 1999; Clark et. al., 1999; Hare and Mantua, 2000; Zhang et al., 2000; Hollowed et al., 2001; Sugimoto et al., 2001; Conners et al., 2002; Hunt et al., 2002; Wilderbuer et al., 2002). During periods of climatic change, some fishes may not be well adapted to dramatic changes over a short time scale (1–10 years), whereas others may proliferate in a more hospitable environment. Key triggers of regime shifts and the extent to which species will respond remain unclear; however, evidence may indicate that species diversity is correlated with primary production in many systems, and the two may be interdependent (Rosenzweig and Abramsky, 1993; Waide et al., 1999). A biodiversity index can be useful for monitoring the stability, health, and productivity of an ecosystem, as well as for aiding management, by tracking exogenous changes and their far reaching effects on species. In the present study, I used biodiversity indexes, reflecting richness and evenness, as indicators of species composition changes and related these changes to regime shift events for the eastern Bering Sea (EBS) shelf.

Methods

Data were synthesized from a continuous 24-year period of standardized groundfish surveys conducted by the Alaska Fisheries Science Center (AFSC) on the EBS shelf from 1979 through 2002; additional estimates from 1975 were included. Biodiversity indexes (species richness and evenness) were calculated from biomass estimates for two fish guilds, flatfish and roundfish, in each of the inner, middle, and outer domains of the EBS shelf. Biodiversity indexes were plotted for each fish group and domains and examined for changes over the study period. The observed changes were correlated with regime shift and ocean climatic change events for the EBS.

Data collection

Gear, station location, sampling procedures, and time of year of the AFSC

eastern Bering Sea continental shelf survey have been standardized since 1982. Prior to 1982, a 400-mesh eastern trawl (smaller than the current 83-112 trawl) was used (Bakkala, 1993). Although the biomass estimates produced by the two trawls are not directly comparable because of unknown catchability differences between the nets, survey data from 1975 and 1979–81 were included in the present analysis because of the importance in the timing of the 1970s regime shift. The biodiversity indexes are based on the relative proportion of the species and therefore are an indicator of assemblage structure.

Data used for this study were collected by the Resource Assessment and Conservation Engineering (RACE) division of the AFSC, which surveys the EBS shelf each summer (May–August). The survey area extends from the Alaska Peninsula north to Nunivak Island and St. Matthew Island, and west to the 200 m shelf break. Trawl hauls were conducted on a grid of 356 fixed stations (20 nmi by 20 nmi) (Fig. 1) during daylight hours. Hauls were towed for 30 min at 3 knots and ranged in depth from 15 m in Bristol Bay to nearly 200 m near the shelf edge. Most trawling was conducted with the AFSC 83-112 eastern trawl (1982–2002), which is a low-opening two-seam trawl with a 26.5-m headrope and 34.1-m cable footrope wrapped with rubber striping and chain hangings that contact the bottom while the trawl is towed (Rose and Walters, 1990).

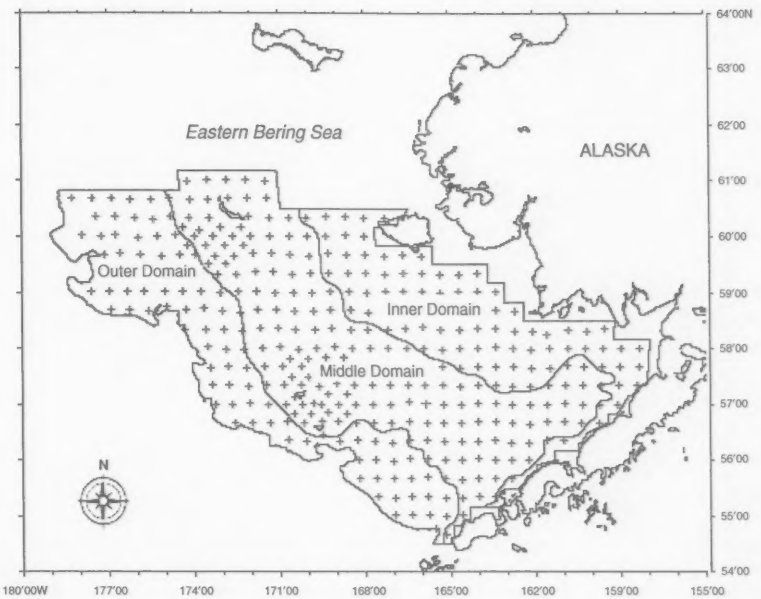


Figure 1

Survey area in the eastern Bering Sea. Crosses represent annual trawl survey stations from 20–200 m. Bathymetry lines delineate the inner, middle and outer domains.

Height and width of the net were measured with an acoustic SCANMAR (Scanmar, Asgardstrand, Norway) or NETMIND (Northstar Technical Inc., St. John's, NF, Canada) net mensuration system, or estimated by using a function that relates trawl widths to tow depths from measured hauls. Each haul was measured with GPS or LORAN to record latitude and longitude data at the start and end of the trawl in order to determine distance fished.

Processing of the catch was done entirely in the field at the time of capture. The entire catch was sorted to species, enumerated, and weighed, or a weighed subsample was used for very large catches.

Catch per unit of area (CPUE) was determined for each trawl station completed during each survey. Biomass estimates for each species were then calculated by expanding the average CPUE for each stratum and then summing over all strata of the total survey area to obtain a biomass estimate in metric tons for each species.

EBS shelf domains and study area

The EBS is composed of three well-defined regions designated as the inner, middle, and outer domains from east to west, respectively, across the shelf (Fig. 1). The three domains are distinct regions characterized by depth, water temperature, current flow, summertime primary production, and species composition (Bakkala, 1993; Schumacher and Stabeno, 1998; Stabeno et al., 2001). Briefly, the inner domain is a relatively shallow (<50 m) and well-mixed warm basin with strong influences from a large coastal area, several major river systems, and a current flow northward along the coast from the Aleutian Islands. The middle domain is a relatively stagnant area of deeper water (50–100 m) that has strong summer water-column stratification and low current flows. The bottom water mass is relatively cold, overlaid by a layer of warmer wind-mixed water. Although characterized as a cold region, the water mass in the middle domain varies from year to year. The outer domain is influenced by the EBS slope region and by upwelling and northward current flow from the Aleutian Islands. This domain is relatively warm when compared to the middle domain (see Hunt et al., 2002, for review). For the purposes of this study the inner domain designation consisted of trawl stations less than 50 m in depth; the middle shelf designation was for trawl stations between 51 and 100 m in depth; and outer domain designation was for trawl stations between 101 and 200 m in depth.

Table 1

Flatfish guild species or species groups used for biodiversity indexes. * indicates the major proportion of the biomass for the group. Superscripts indicate the primary domains where each species occurs during the summertime AFSC survey. I=inner domain, M=middle domain, O=outer domain.

Group name	Scientific name
Pacific halibut	*Hippoglossus stenolepis*^{IMO}
Flathead/Bering flounder	**Hippoglossoides elassodon* and *H. robustus*^{IMO}
Greenland turbot	*Reinhardtius hippoglossoides*^{O}
Arrowtooth/Kamchatka flounders	**Atheresthes stomias* and *A. evermanii*^{MO}
Starry flounder	*Platichthys stellatus*^{IM}
Alaska plaice	*Pleuronectes quadrituberculatus*^{IM}
Northern/southern rock sole	**Lepidopsetta polyxystra* and *L. bilineata*^{IMO}
Longhead dab	*Limanda proboscidea*^{I}
Yellowfin sole	*Limanda aspera*^{IM}
Rex sole	*Glyptocephalus zachirus*^{O}
Oher flatfish	**Isopsetta isolepis*^{IM}, **Limanda sakhalinensis*^{MO}, *Psettichthys melanostictus, Microstomus pacificus*

Fish assemblages differed noticeably among the inner, middle, and outer domains in the EBS; many species (sculpins, poachers, and eelpouts) primarily inhabited a single domain and many flatfishes and skates inhabited all three domains (Kaimmer[1]; Kinder and Schumacher, 1981; Smith and Bakkala, 1982: Tables 1 and 2). Because of the distinct domain environments and assemblages, the flatfish and roundfish species groups were analyzed by inner, middle, and outer domains separately and all domains were combined to detect the influence of climate change on each assemblage.

Species groups

The taxonomic level of species identification for the entire survey period (1975–2002) has varied because of an incomplete knowledge of species characters and because of survey time constraints at-sea. Historically, survey goals were to obtain fisheries data on commercially or potentially commercially important species, and limited effort was put forth for identification of other species. However, given more efficient technologies, better identification field guides, and increased focus on noncommercial species, species identification has improved and is approaching the current extent of taxonomic knowledge.

Efforts in recent years (1998–2002) have increased our knowledge of current species distributions in the EBS shelf region. To assess species-identification confidence over the study period, current species distributions from the AFSC survey and primary literature not associated with AFSC survey data, as well as historical fish collections of selected taxonomic groups (University of Washington Fish Collection, Oregon State University Fish Collection, and Auke Bay Fish Collections) were examined. After this assessment, it was subjectively determined whether species in this study were identified correctly throughout the study period. Species that were determined to be distinguishable and correctly identified were examined as individual species, and species that were possibly misidentified were grouped at a higher taxonomic level for this study. The resulting list of species or species groups includes the greatest number of fish taxa (Tables 1 and 2).

At least 15 flatfish species (within the order Pleuronectiformes) and more than 75 species of fish and elasmobranchs not of the order Pleuronectiformes were recorded during the AFSC summer surveys (1975–2002) on the EBS shelf (20–200 m). Although flatfish make up about 16% of the total number of fish species, they contribute approximately 50% of the biomass of all fish combined (2001 and 2002 AFSC EBS survey estimates). Walleye pollock (*Theragra chalcogramma*) and Pacific cod (*Gadus macrocephalus*) represent approximately 42% of the survey biomass, and 8% of the biomass that included all other roundfish. Because of the extremely large biomass of walleye pollock and Pacific cod, the biodiversity indexes used are uninformative when walleye pollock and Pacific cod are included owing to the "swamping" effect by their relatively large biomasses when compared to those of other species. To account for this, two species guilds were defined: flatfish and roundfish. The flatfish guild included all Pleuronectiformes recorded from the EBS survey and comprised 11 species or species groups (Table 1). The roundfish

[1] Kaimmer, S. M., J. E. Reeves, D. R., Gunderson, D. R., Smith, G. B., and R. A. Macintosh. 1976. Baseline information from the 1975 OCSEAP survey of the demersal fauna of the eastern Bering Sea. *In* Demersal fish and shellfish resources of the eastern Bering Sea in the baseline year 1975 (W. T Pereyra, J. R. Reeves, and R. G. Bakkala, eds.), p. 157–367. NWAFC Processed Report. Alaska Fisheries Science Center, National Marine Fisheries Service, NOAA, 7600 Sand Point Way N.E. Seattle WA 98115.

Table 2

Roundfish guild species or species groups used for biodiversity indexes. * indicates the major proportion of the biomass for the group. Superscripts indicate the primary domains where each species occurs during the summertime AFSC survey. I=inner domain, M=middle domain, O=outer domain. IMO=inner, middle, and outer domains.

Group name	Scientific name
Lamprey	*Lampetra tridentata*
Skate	**Bathyraja parmifera*^{IMO}, **B. interrupta*^{O}, *B. aleutica, B. taranetzi, Raja binoculata*
Shark	**Somniosus pacificus*^{O}, *Squalus acanthias, Lamna ditropis*
Other poachers	**Aspidophoroides bartoni*^{O}, *Bathyagonus alascanus, Leptagonus leptorhynchus, Pallasina barbata, Percis japonicus*
Sawback poacher	*Sarritor frenatus*^{MO}
Sturgeon poacher	*Agonus acipenserinus*^{IM}
Bering poacher	*Ocellus dodecaedron*^{I}
Bering wolffish and wolfeel	*Anarhichas orientalis* and *Anarrhichthys ocellatus*
Searcher	*Bathymaster signatus*^{O}
Other sculpins	*Artediellus pacificus, Leptocottus armatus, Enophrys* spp., *Psychrolutes paradoxus,Blepsias bilobus, Nautichthys pribilovious, Icelinus* spp.
Staghorn sculpin	**Gymnocanthus pistilliger*^{I}, **G. galeatus*^{O}, *G. detrisus*
Darkfin sculpin	*Malacocottus zonurus*^{O}
Yellow Irish lord	*Hemilepidotus jordani*^{I}
Butterfly sculpin	*Hemilepidotus papilio*^{M}
Triglops sculpins	**Triglops scepticus*^{O}, **T. pingeli*^{IM}, *T. macellus, T. forficatus*
Myoxocephalus sculpins	**Myoxocephalus jaok*^{I}, **M. polyacanthocephalus*^{MO}, *M. verrucosus*^{M}
Spinyhead sculpin	*Dasycottus setiger*^{O}
Bigmouth sculpin	*Hemitripterus bolini*^{O}
Icelus sculpins	**Icelus spiniger*^{O} and **I. spatula*^{IM}
Pacific sandfish	*Trichodon trichodon*^{I}
White-spotted greenling	*Hexagrammos stelleri*^{I}
Snailfish	**Liparis gibbus*^{MO}, **Careproctus rastrinus*^{MO}, *C. cyclospilus*
Smooth lumpsucker	**Aptocyclus ventricosus*^{O}, *Eumicrotremus orbis*
Pricklebacks	**Lumpenus maculata*^{IMO}, **L. sagittae*^{IMO}, *L. fabricii, L. medius, Poroclinus rothrocki*
Other eelpouts	**Lycodes* spp. and *Gymnelus* spp.
Marbled eelpout	*Lycodes raridens*^{I}
Wattled eelpout	*Lycodes palearis*^{MO}
Shortfin eelpout	*Lycodes brevipes*^{O}
Pacific tomcod	*Microgadus proximus*
Saffron cod	*Eleginus gracilis*^{I}
Arctic cod	*Boreogadus saida*^{M}
Pacific sand lance	*Ammodytes hexapterus*
Pacific herring	*Clupea pallasii*^{IM}
Eulachon	*Thaleichthys pacificus*^{O}
Capelin	*Mallotus villosus*^{I}
Rainbow smelt	*Osmerus mordax*^{IMO}
Salmon	*Oncorhynchus* spp.
Sablefish	*Anoplopoma fimbria*^{O}
Atka mackerel	*Pleurogrammus monopterygius*
Prowfish	*Zaprora silenus*^{O}
Rockfish	**Sebastes alutus*^{O}, *S. polyspinis, S. ciliatus*

guild included 40 species or species groups and excluded walleye pollock and Pacific cod (Table 2). The two guilds devised were subjective; however, owing to their distinctive biomass and life history trends, the members of the flatfish guild were examined separately from all other species.

Biodiversity indexes

Biodiversity measures were calculated by using Ludwig and Reynolds's (1988) recommendations for species richness and evenness. Richness and evenness are considered robust measures and allow one to use biomass proportions for biodiversity estimations. The richness index used was as follows:

$$Richness\ index = e^{H'}$$

$$\text{where } H' = \sum_{i=1}^{S}\left[\left(\frac{n_i}{n}\right)\ln\left(\frac{n_i}{n}\right)\right]$$

and n_i = the biomass of individuals belonging to the ith of S species in the sample; and
n = the total summed biomass for the entire guild for a given year (Ludwig and Reynolds, 1988).

The evenness index is the "modified Hill's ratio" (Alatalo, 1981) which is

$$Evenness = \frac{\left(\frac{1}{\lambda}\right)-1}{e^{H'}-1},$$

where $\lambda = -\sum_{i=1}^{S} p_i^2$, where $Pi = \frac{n_i}{N}$ and n_i is the biomass of the ith species and N is the total biomass of all S (species) in the guild (Ludwig and Reynolds, 1988).

Biomass estimates obtained from the surveys were used to calculate biodiversity values for both flatfish and roundfish guilds on an annual basis. Biodiversity values calculated were used as an index of the relative proportion of species in each guild and allowed a direct comparison from year to year of the most abundant species. Species richness indicated the effective number of species that are influencing the index (Hill, 1973; Ludwig and Reynolds, 1988). A higher index indicates a larger number of predominant species in the assemblage (i.e., higher diversity). The evenness index determines the distribution of the biomass proportions of the more abundant species among the species in the guild. As the evenness index approaches zero, the biomass proportion of a single species dominates the guild; as the evenness index approaches one, there is less of a single dominant species and the biomass proportions are shared more equally among many species in the guild (Hill, 1973; Ludwig and Reynolds, 1988).

A piecewise linear model (Neter et al., 1996) was applied to the biodiversity indexes using S-Plus (vers. 6.1, Insightful Corporation, Seattle, WA) to determine distinct breaks or inflection points in the linear data trends. The piecewise model finds a "knot," or inflection point, that breaks the data set into two periods by using the least sum of squared residuals and the highest R^2 for the two-line model to determine the best fit lines to the data. Linear regression models were then applied to the data sets by using the recommended inflection point as the breaking point for the two lines. The years covered for each best fit line, as well as the accompanying linear statistics for the individual lines and the piecewise linear model for the best-fit model are presented in Table 3. The indexes calculated for survey year 1975 are included for reference (denoted as an X on the diversity index graphs) but were not included in the linear regression models because of the time gap from 1975 to 1979 and the lack of confidence in the standardization of the 1975 survey compared with later surveys (Bakkala, 1993).

Results

Flatfish biomass

Biomass estimates for the flatfish guild showed an increase from the late 1970s until the early 1990s, and then an overall slight decline between 1999 and 2002 for all domains (Fig. 2). Species that represented a large portion that showned an increase in biomass estimates over the study period included northern and southern rock sole (*Lepidopsetta polyxystra and L. bilineata,* spp. undetermined), arrowtooth and Kamchatka flounder (*Atheresthes stomias* and *A. evermanni,* spp. undetermined), flathead sole and Bering flounder (*Hippoglossoides elassodon* and *H. robustus,* spp. undetermined), and Pacific halibut (*Hippoglossus stenolepis*). Flatfish species with declining biomass estimates included the yellowfin sole *(Limanda aspera)* and Greenland turbot (*Reinhardtius hippoglossoides*), and a decline in lesser abundant species such as longhead dab (*Limanda proboscidea*) and Alaska plaice (*Pleuronectes quadrituberculatus).* The inner and middle domains were dominated by northern rock sole, yellowfin sole, Alaska plaice, and flathead sole and Bering flounder (spp. undetermined), whereas main portions of the outer shelf comprised arrowtooth and Kamchatka flounder (spp. undetermined), flathead sole and Bering flounder (spp. undetermined), Pacific halibut, and northern and southern rock sole (spp. undetermined).

Roundfish biomass

Roundfish biomass increased from the 1970s to the 1980s and then declined steadily from the early 1990s through 2002 for all domains combined (Fig. 2). Among roundfish species there has been a decline since the 1970s and 1980s in many middle domain species, such as yellow Irish lord (*Hemilepidotus jordani*), butterfly sculpin (*H. papilio*),

Table 3

Statistics of the linear regression models of biodiversity indexes for flatfish and roundfish guilds for the eastern Bering Sea shelf domains. The years encompassing each time period and the piecewise model r^2 for the two-line model are included.

Domain	Index	Years	*n*	Intercept	Slope	*P*-value	r^2	Standard error of estimate	r^2 of piecewise model
Flatfish guild									
Inner	Richness	1979–1993	15	–112.9280	0.0579	0.0000	0.8797	0.0993	0.8850
		1994–2002	9	3.9168	–0.0008	0.9645	0.0303	0.1270	
Middle	Richness	1979–1988	10	–358.4240	0.1821	0.0001	0.8829	0.2130	0.8640
		1989–2002	14	7.8482	–0.0020	0.9171	0.0940	0.2887	
Outer	Richness	1979–2002	24	–2.8281	0.0030	0.6875	0.7493	0.2533	
Combined	Richness	1979–1989	11	–327.9090	0.1666	0.0000	0.8740	0.2212	0.9350
		1990–2002	13	6.1232	–0.0012	0.8866	0.1932	0.1150	
Inner	Evenness	1979–1990	12	–48.3712	0.0247	0.0000	0.9291	0.0258	0.9610
		1991–2002	12	–0.0349	0.0005	0.6927	0.0163	0.0161	
Middle	Evenness	1979–1987	9	–61.6390	0.0314	0.0003	0.8663	0.0361	0.8660
		1988–2002	15	3.7805	–0.0015	0.5159	3.3171	0.0375	
Outer	Evenness	1979–2002	24	1.8798	–0.0006	0.6184	0.0115	0.0392	
Combined	Evenness	1979–1988	10	–59.8421	0.0305	0.0000	0.9318	0.0265	0.9630
		1989–2002	14	2.0678	–0.0007	0.4732	0.0437	0.0136	
Roundfish guild									
Inner	Richness	1979–2002	24	–5.0326	0.0040	0.8287	0.2175	0.6197	
Middle	Richness	1979–1986	8	865.6880	–0.4338	0.0119	0.6789	0.7895	0.9040
		1987–2002	16	203.6660	–0.1006	0.0000	0.7196	0.3095	
Outer	Richness	1979–1986	8	908.1290	–0.4564	0.0011	0.8511	0.5051	0.8690
		1987–2002	16	–65.9678	0.0340	0.0762	0.2075	0.3276	
Combined	Richness	1979–1986	8	1535.3000	–0.7713	0.0003	0.9072	0.6527	0.9410
		1987–2002	16	81.4226	–0.0394	0.1204	0.1634	0.4390	
Inner	Evenness	1979–1990	12	–14.9002	0.0078	0.3003	0.1066	0.0853	0.5620
		1991–2002	12	–32.0753	0.0164	0.0057	0.5504	0.0561	
Middle	Evenness	1979–1990	12	51.5981	–0.0257	0.0001	0.7779	0.0518	0.9210
		1991–2002	12	20.3337	–0.0099	0.0002	0.7694	0.0206	
Outer	Evenness	1979–1987	9	66.1545	–0.0331	0.0024	0.7550	0.0552	0.7150
		1988–2002	15	–16.5892	0.0085	0.0110	0.4028	0.0482	
Combined	Evenness	1979–1989	11	46.7485	–0.0233	0.0003	0.7782	0.0434	0.8630
		1990–2002	13	1.1549	–0.0003	0.8818	0.0021	0.0306	

armorhead sculpin (*Gymnocanthus galeatus*), snailfishes (*Liparis gibbus* and *Careproctus rastrinus*), marbled eelpout (*Lycodes raridens*), and wattled eelpout (*Lycodes palearis*), and in outer domain species such, as shortfin eelpout (*Lycodes brevipes*) and sablefish (*Anoplopoma fimbria*). There has been a increase in large sculpins, including the bigmouth sculpin (*Hemitripterus bolini*) and the great sculpin (*Myoxocephalus polyacanthocephalus*), in the outer domain. The biomass of skates predominantly the Alaska skate (*Bathyraja parmifera*), increased considerably from 1979 through 1990, followed by a prolonged period of little change from 1990 through 2002.

Flatfish biodiversity

Eleven species or species groups were used for biodiversity indexes for the flatfish guild (Table 1). All 11 species are rarely found together, and within each shelf domain the major portion of the assemblage comprises 3–5 species. Figure 3 shows the change in biodiversity indexes from 1975 to 2002 for each domain separately and all domains combined. The piecewise model shows a period of inflection for evenness and richness indexes for flatfish in the inner and middle domains that occurred between 1987 and 1993, with the average year of inflection being 1990 (Fig. 3, Table 3). The outer domain for flatfish showed little change in evenness and richness throughout the 24-year study period. Three species dominated the entire period, with somewhat evenly distributed biomass proportions.

Roundfish biodiversity

Forty-one species or species groups were used in the roundfish biodiversity analysis (Table 2). The evenness and species richness index for the roundfish group

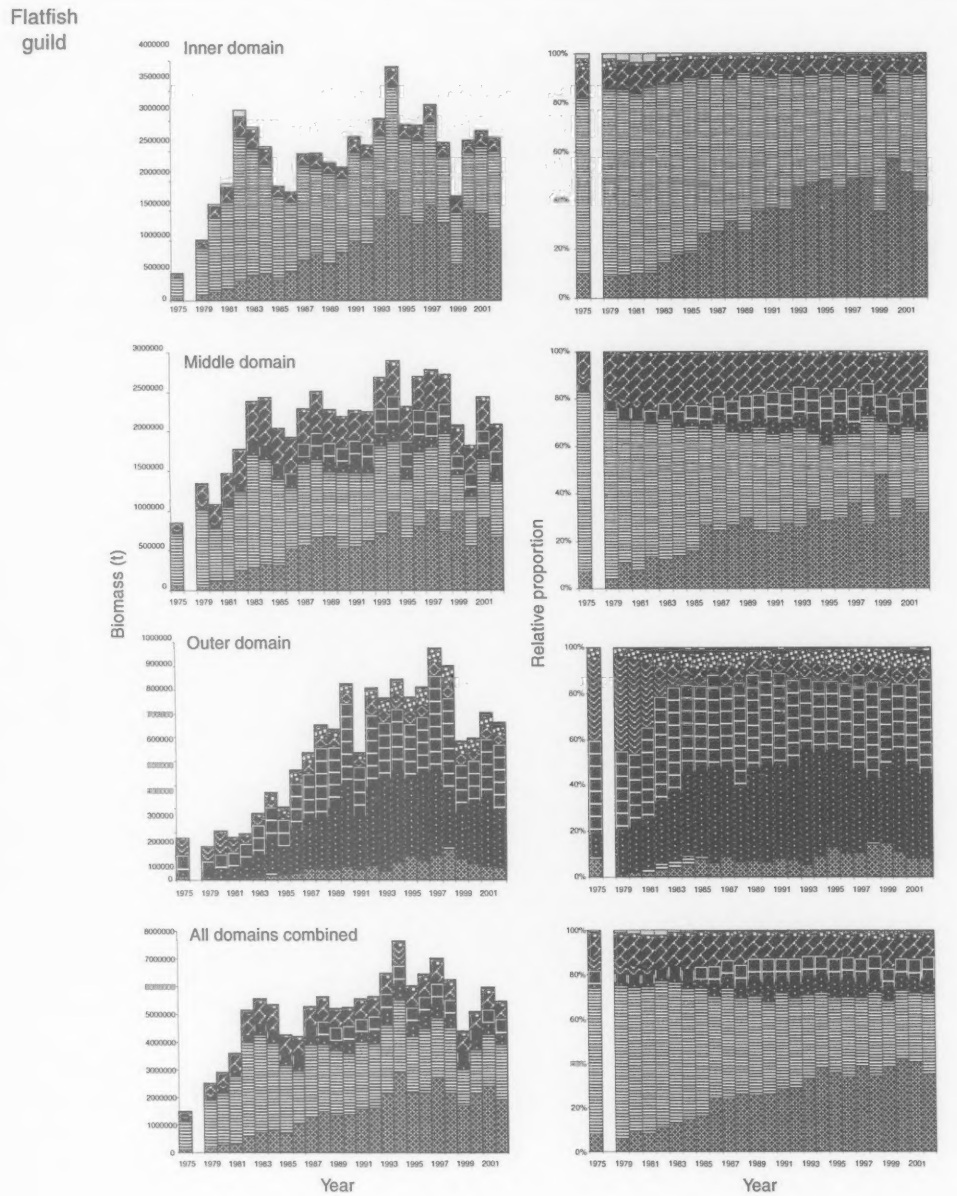
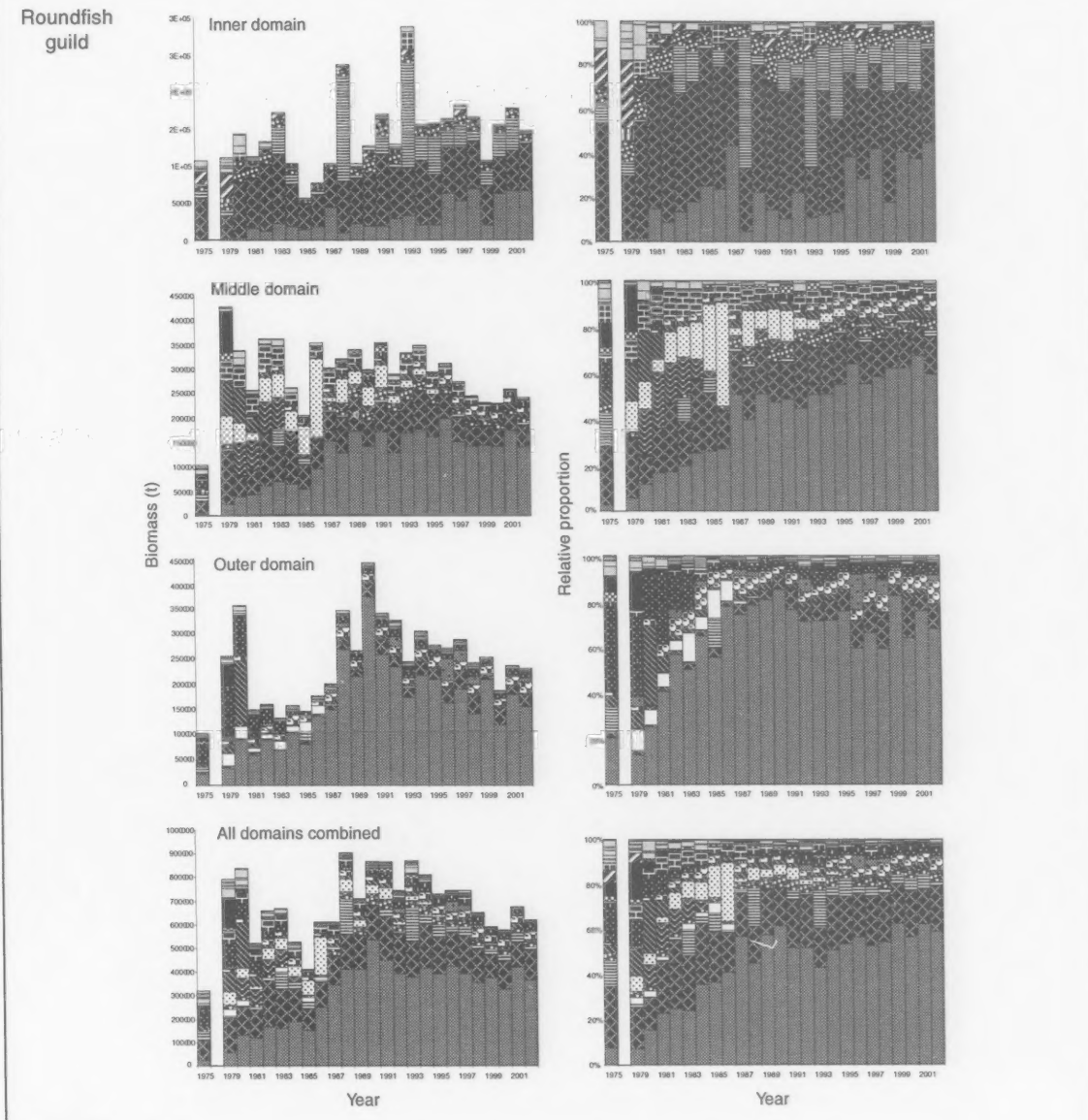


Figure 2

Biomass estimates for flatfish guild and roundfish guild (left panel), and relative proportion of each species (right panel) for inner, middle, outer, and all three domains combined, respectively.



Roundfish guild

- Atka mackeral
- prowfish
- pricklebacks
- salmon
- hagfish and lampreys
- sharks
- arctic cod
- Pacific tomcod
- rainbow smelt
- eulachon
- Pacific sand lace
- Pacific sandfish
- other sculpins
- *Icelus* sculpins
- spinyheaded sculpin
- *Triglops* sculpins
- darkfin sculpin
- armorhead sculpin
- Bering wolffish and wolfeel
- Bering poacher
- sawback poacher
- other poachers
- searcher
- lumpsuckers
- capelin
- saffron cod
- other eelpouts
- snailfishes
- white spotted greenling
- yellow Irish lord
- shortfin eelpout
- rockfish
- bigmouth sculpin
- wattled eelpout
- butterfly sculpin
- marbled eelpout
- sturgeon poacher
- sablefish
- Pacific herring
- *Myoxocephalus* sculpins
- skates

Figure 2 (continued)

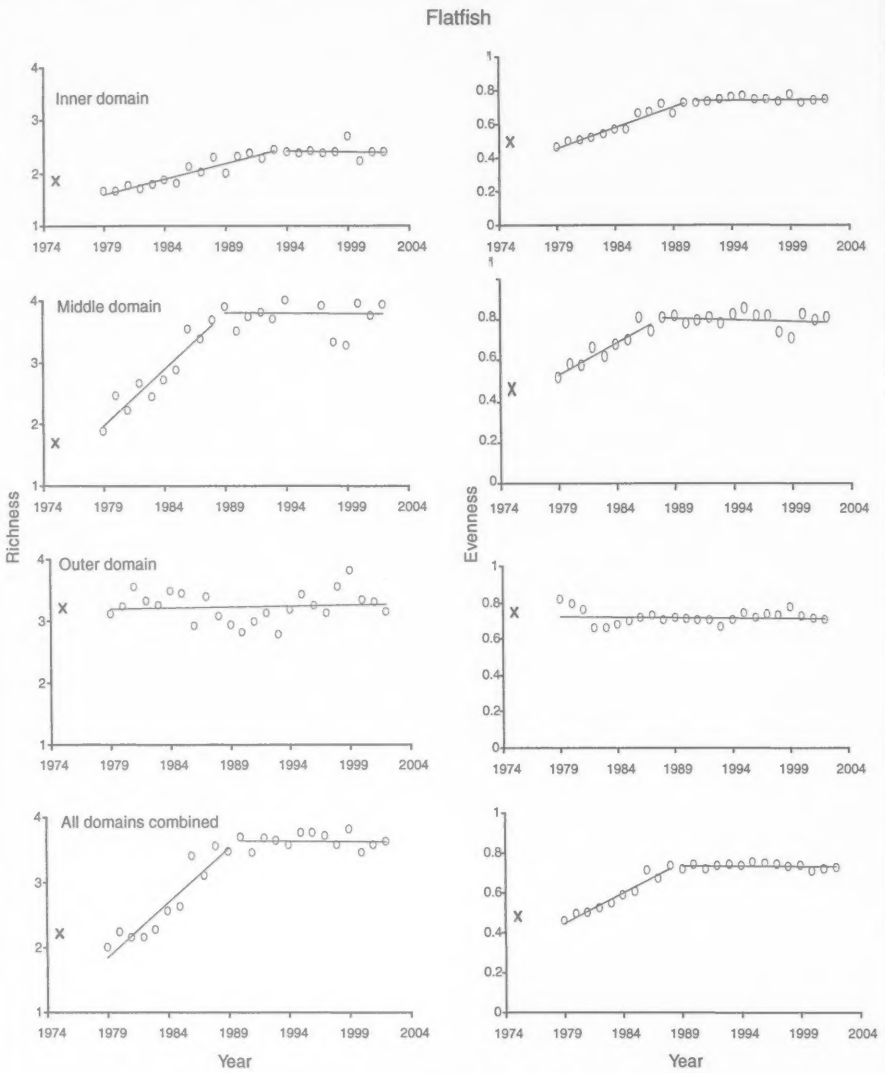


Figure 3

Biodiversity indexes (richness left, evenness right) for the flatfish guild determined with two linear models (or a single model) separated at the inflection point derived from the piecewise linear model. The graphs represent the inner, middle, outer, and all domains combined, respectively. The X represents biodiversity index for survey year 1975 which was not included in linear models or the piecewise model because of a gap in the time series.

is plotted for each shelf domain and for all domains combined. The inner domain richness index revealed a steady level of approximately 2–3 dominant species or species groups (*Myoxocephalus* sculpins, skates, and Pacific herring) over the 24-year period. The gradual increase in skates and slight decline in the plain sculpin caused an increase in the evenness index to somewhat even proportions of the three dominant species in the inner domain, from 7 to 2 dominant species over the 24-year period, and evenness declined by about one-half (Fig. 4). The increase in skates and decline of many inner domain species from 1975 through the early 1990s resulted in an assemblage dominated by two species groups, *Myoxocephalus* sculpins and skates (Fig. 2).

The outer domain showed a dramatic decline in richness and evenness from 1975 to the mid-1980s and

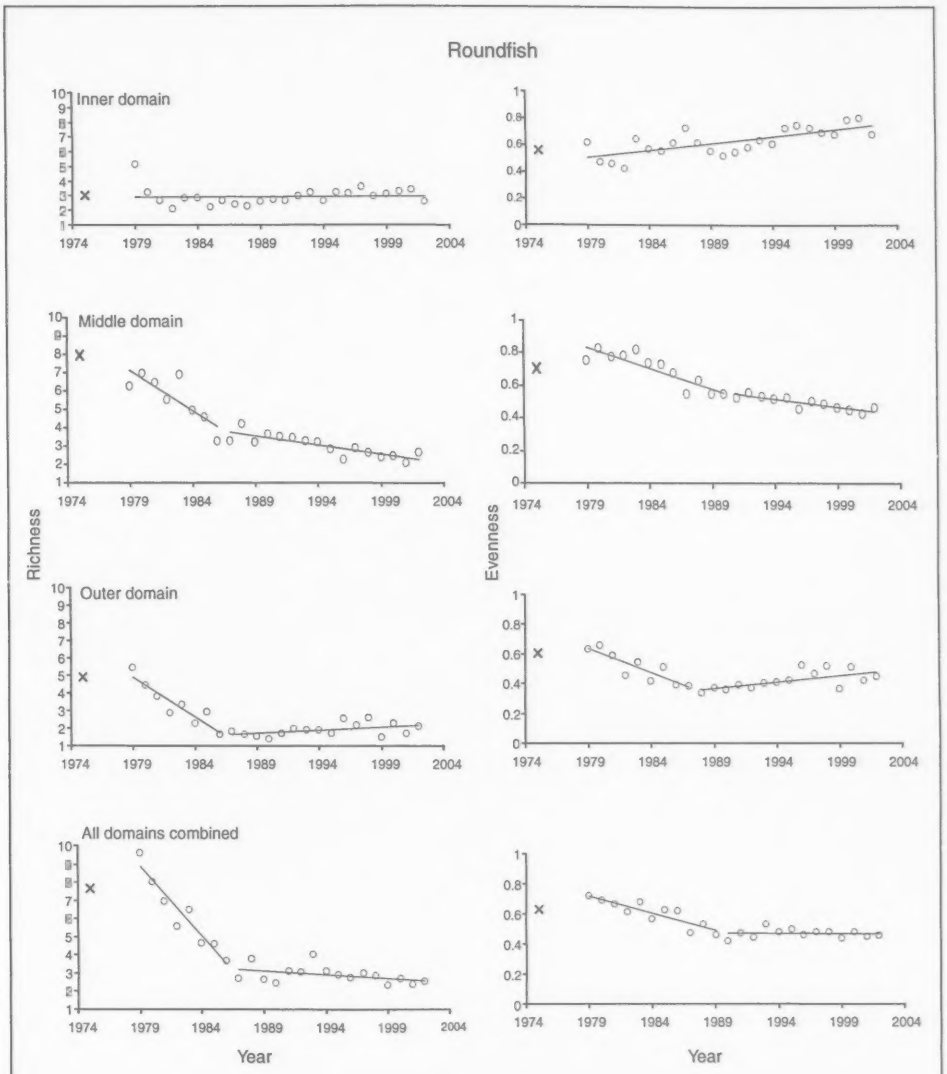


Figure 4

Biomass estimates of roundfish guild (left panel), and relative proportion of each species (right panel) for the inner, middle, outer, and all domains combined, respectively.

then a slight increase through 2002. A decrease in the number of dominant species, such as sablefish, wattled and shortfin eelpouts from 1979 to 1986, and a large increase of skate biomass from 20% in 1975 to approximately 75% of the total biomass by 1986, explain the index decline during this same period as the skate biomass dominated. After 1986 the skate populations stabilized somewhat to a slight decline which accounts for the slight increase in evenness and richness as other species gained a larger percentage of the total biomass.

All indexes for the roundfish guild, except for inner domain richness, indicated two distinct linear periods. Inflection points ranged from 1986 to 1990 and 1988 was an average inflection year for the roundfish guild.

Discussion

Periods of climatic change have been shown to instill long-term changes in the North Pacific ecosystem (Hare

and Mantua, 2000). Biodiversity indexes are a robust measure for large ecosystem monitoring and possible indicators of resulting regime shift phenomenon from climatic change based on an assemblage's long-term responses. Hare and Mantua (2000) summarized over 100 studies from the eastern North Pacific identifying two distinct periods of change or "regime shifts" in 1977 and 1989. Recently, Stabeno et al. (2001) and Hunt and Stabeno (2002) suggested the possibility of a third period of significant climatic change in the late 1990s for the northeastern Pacific. Although environmental changes may be readily identified, the ecosystem fauna may respond more slowly, or in ways not immediately obvious; however the changes may be persistent and far reaching. According to survey data, the last 20 years have proven to be a very hospitable environment for many flatfish species in the EBS, where populations such as northern rock sole and arrowtooth and Kamchatka flounder have rapidly expanded since the late 1970s. Biodiversity indexes are significantly higher than they were 20 years ago for flatfish as a group and have remained high and unchanged. The biodiversity indexes for flatfish guild corroborate the timing of the strong regime shift reported in the late 1980s. An inflection around 1990 is the strongest evidence of a regime shift from the biodiversity indexes. It would be expected that a purely biological response to a climatic event may lag by a time period, perhaps accounting for a later year than previously reported.

Survey data indicate that many roundfish species have not fared as well as flatfish species and biodiversity indexes are significantly lower now than 20 years ago. The roundfish guild has undergone a significant reorganization in which a large group of species have declined and a single species has become dominant, causing biodiversity to be suppressed. The regime shift is reflected in the roundfish biodiversity around 1988, which agrees with the results of other reported studies.

Although EBS productivity has increased through the 1970s and 1980s and remained high through the 1990s, the trend in the ecosystem is towards fewer or single dominant species. The decline of many nonexploited species in the EBS is difficult to explain with the current regime shift theories. Unfortunately, a lack of information on the life history of the large number of species, such as poachers, sculpins, eelpouts, and skates in the EBS hinders a complete understanding of recruitment success or failure.

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Abstract—A new species of the cottid genus *Triglops* Reinhardt is described on the basis of 21 specimens collected in Aniva Bay, southern Sakhalin Island, Russia, and off Kitami, on the northern coast of Hokkaido, Japan, at depths of 73–117 m. Of the ten species of *Triglops* now recognized, the new species, *Triglops dorothy*, is most similar to *T. pingeli* Reinhardt, well known from the North Atlantic and North Pacific oceans and throughout coastal waters of the Arctic. The new species differs from *T. pingeli* in a combination of morphometric and meristic characters that includes most importantly the number of dorsolateral scales; the number of oblique, scaled dermal folds below the lateral line; and the number of gill rakers.

Triglops dorothy, a new species of sculpin (Teleostei: Scorpaeniformes: Cottidae) from the southern Sea of Okhotsk

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Sculpins of the teleost family Cottidae are nearly ubiquitous in cold-water benthic habitats of the Northern Hemisphere, comprising nearly 200 species in the North Pacific alone (Yabe and Nakabo, 1984; Sheiko and Federov, 2000; Mecklenburg et al., 2002; Love et al., 2005), where they are found in almost every benthic habitat from the intertidal to the upper continental slope. Many species are preyed upon by larger fishes and marine mammals (Browne et al., 2002), and are themselves predators primarily of smaller fishes and crustaceans (e.g., Tokranov, 1998; Hoff, 2000; Tokranov and Orlov, 2001). Although many members of the family are also commonly found in bycatch of commercial fisheries (Stevenson, 2004; Orlov, 2005), the systematics and life histories of most species are poorly known (Nelson, 1994; Hoff, 2000; Hoff, 2006) and new species continue to be described (e.g., Yabe, 1995; Yabe and Maruyama, 2001; Yabe et al., 2001). A more complete understanding of the diversity of the family is necessary to understand the role of cottids in the dynamics of North Pacific ecosystems.

The cottid genus *Triglops* Reinhardt (1830), including *Prionistius* Bean (1884), *Elanura* Gilbert (1896), and *Sternias* Jordan and Evermann (1898) as junior synonyms, contains 19 nominal species and subspecies, of which nine are currently recognized as valid (Pietsch, 1993). Members of the genus are characterized most strikingly by having a small head, a narrow, elongate body and a slender caudal peduncle, a long anal fin containing 18–32 rays, pelvic fins with a single spine and three soft rays, branchiostegal membranes united on the ventral midline but lying free from the isthmus, and scales below the lateral line modified to form discrete rows of tiny serrated plates that lie in close-set, oblique dermal folds. The species are generally distributed in rather deep water (primarily between 18 and 600 m, but specimens have been taken more or less on the surface and as deep as 930 m; Andriashev, 1949; Hart, 1973; Fedorov, 1986) throughout cold-water, continental shelf or slope regions of the North Pacific, North Atlantic, and Arctic oceans. All appear to feed primarily on small planktonic and benthic invertebrates (Andriashev, 1949; Fedorov, 1986). Spawning takes place from late summer to winter; the eggs are demersal (Andriashev, 1949; Musick and Able, 1969; Fedorov, 1986).

Triglops was first proposed by Johannes Reinhardt (1830), based on a single specimen from West Greenland, but the type species *T. pingeli*

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was not named until 1837, after Reinhardt received two additional specimens, also from West Greenland. During the next 130 years, 18 additional species or subspecies were described, but little work of a comparative nature was done. Schmidt (1930) reviewed some North Pacific specimens of the group, and later (Schmidt, 1950) discussed species identified in his work on the ichthyofauna of the Sea of Okhotsk. Taranets (1941) placed the genus, together with *Sternias*, in its own subfamily. The Arctic and North Pacific species of *Triglops* were studied by Jensen (1944), Andriashev (1949, 1954), and McAllister (1963), and a world-wide revision of the genus was subsequently published by Pietsch (1993).

Within the material of *Triglops* examined by Pietsch (1993) are 19 specimens that were originally recognized by Charles Henry Gilbert as new to science (see "Discussion" below). Labeled as such, but left unpublished by Gilbert, Pietsch (1993) came to the same conclusion, citing a lack of agreement of these specimens with the diagnosis of any of the nine recognized species of the genus. However, still not totally convinced that the specimens represented a new species, Pietsch (1993) again postponed a formal description. Labeled *Triglops* sp. and set aside at that time, these specimens are now re-examined in light of the discovery of two additional specimens, and evidence is provided to formally recognize the material as a new species.

Methods and materials

Standard length (SL) is used throughout unless indicated otherwise. Measurements are expressed in percent of standard length or in percent of head length (HL). Methods used for taking counts and measurements follow those described and figured by Miller and Lea (1972:7–12), with the following additions: interorbital width is measured between lateral margins of the frontal bones at their narrowest widths; head depth is measured at mid-orbit; first infraorbital pore width is measured at the greatest width, anterior to posterior; all gill-raker counts are taken from the first arch; dorsolateral scale-row counts include all scales (or fused clusters of scales) with enlarged plate-like bases; lateral-line scale counts also include scales that occasionally extend beyond the posterior margin of the hypural plate. Pectoral-fin rays are counted from dorsal to ventral. Counting the oblique dermal folds on the ventral half of the trunk presents a special problem. Because many dermal folds (the number depending on the species), which originate along the lateral line, terminate irregularly before reaching the ventral midline, counts can be considerably higher and more highly variable (than the values reported in the present study) if made along a transect close to the lateral line; thus, it is important to count the dermal folds along a transect beginning beneath the pectoral fin and continuing along, close to and strictly parallel with, the base of the anal fin (see Pietsch, 1993:337, Fig. 1). Dermal folds of the breast are counted along the ventral mid-line. Symbolic codes for institutions are those provided by Leviton et al. (1985).

Statistical significance was assessed by ANOVA; all differences at $P<0.05$ were considered significant. To aid in the discrimination of the new species and its closest ally, *T. pingeli*, a standard principal component analysis (PCA) was conducted on meristic characters, and a sheared PCA was conducted on morphometric characters for a size-free analysis. The analyses were conducted on the correlation matrix of raw meristic data and the covariance matrix of log-transformed raw morphometric data. Differences between species were illustrated by plotting principal component (PC) 1 against PC2 of the meristic analysis, sheared PC2 against sheared PC3 of the morphometric analysis, and sheared morphometric PC2 against the standard meristic PC1.

Following the PCA, a linear discriminant function analysis (DFA) was conducted on morphometric and meristic data to establish the relative significance of those characters in distinguishing the species. Morphometric data were standardized by dividing by standard length. Each character was tested to verify assumptions of multivariate normality and for statistical significance; those found to be distributed nonnormally or that were nonsignificant were eliminated from further analysis. The robustness of the DFA was tested by conducting a leave-one-out cross-validation procedure. Statistical analyses were performed with S-Plus (vers. 6.1 for Windows, 2002, Insightful, Inc., Seattle, WA) and SPSS (vers. 11.5.1 for Windows, 2002, SPSS Inc., Chicago, IL).

Systematics

Triglops dorothy Pietsch and Orr, n. sp.

Dorothy's sculpin

Figures 1–4, Tables 1–3

Triglops sp.: Pietsch, 1993:379 (probably an undescribed species, description postponed).

Holotype

USNM (U.S. National Museum of Natural History, Washington, D.C.) 74578, female, 155 mm, *Albatross* Station 5013, Aniva Bay, 46°17′N, 143°09′E, 3-m Agassiz trawl, 77 m, substrata unknown, 25 September 1906.

Paratypes

Twenty specimens, 15 males (72.5–148.0 mm), 5 females (68.5–143.0 mm). The following material was collected by the U. S. Fish Commission Steamer *Albatross* in or adjacent to Aniva Bay, Sakhalin Island, Russia, with a 3-m Agassiz trawl: CAS-SU 22305, 3 females (68.5–133.0 mm), station 5007, 46°03′N, 142°31′E, 77 m, green mud, fine gray sand, 24 September 1906. USNM 74575, 2 males (72.5–80.0 mm), station 5006, 46°04′N, 142°29′E, 79 m, green mud, fine gray sand, 24 September 1906;

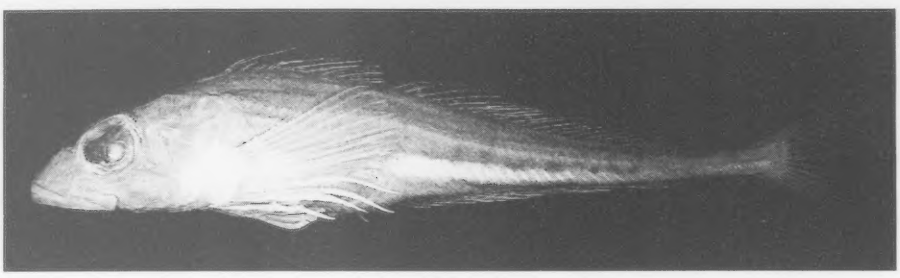

Figure 1

Triglops dorothy n. sp., holotype, female, 155 mm, USNM 74578.

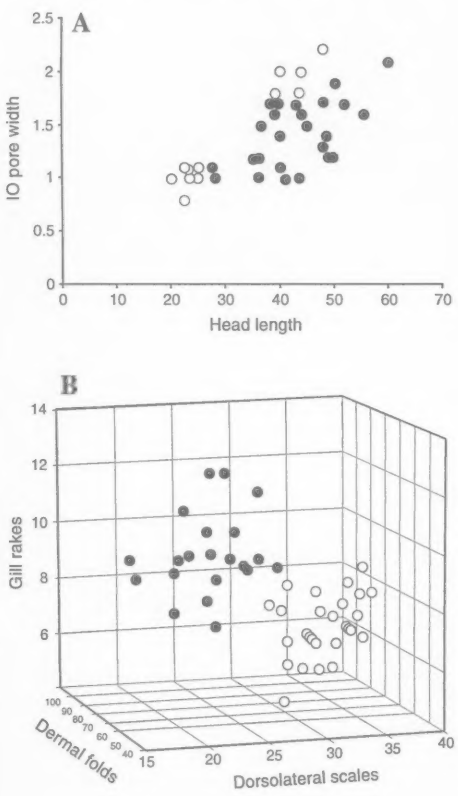

Figure 2

Plots of significant characters differentiating *Triglops dorothy* n. sp. (○) from *Triglops pingeli* (●) in the Sea of Okhotsk: (**A**) width of first infraorbital (IO) pore versus head length; (**B**) Counts of gill rakers versus dermal folds versus dorsolateral scales.

USNM 74576, 3 males (73.0–77.5 mm), 1 female (83.0 mm), station 5008, 46°07′50″N, 142°37′20″E, 73 m, green mud, fine gray sand, 24 September 1906; USNM 74577, female (143.0 mm), station 5016, 46°44′30″N, 143°45′E, 117 m, brown mud, fine black sand, rock, clay, 25 September 1906; USNM 74579, 8 males (73.5–80.0 mm), station 5005, 46°04′40″N, 142°27′30″E, 77 m, green mud, fine gray sand, 24 September 1906. The following material was collected from off Kitami, Hokkaido, Japan, September 1964: HUMZ 41524, 1 male (132.0 mm); HUMZ 41525, 1 male (148.0 mm).

Diagnosis

A species of *Triglops* distinguished from all other described members of the genus in having the following combination of character states: interorbital width 8.9–11.5% HL; caudal fin truncate to slightly rounded; dorsolateral scales well developed, 18–31; lateral-line scales 47–50; transverse dermal folds of breast 4–10; oblique dermal folds 67–99; dorsal-fin rays 23–25; pectoral-fin rays 17–19; gill rakers 7–12; first infraorbital pore length 3.6–5.0% HL; peritoneum everywhere unpigmented or at most with few, widely spaced melanophores.

Description

Body relatively compressed, tapering gradually posteriorly (Fig. 1); head compressed, length 28.4–32.3% SL (Table 1), depth 11.3–13.5% SL; snout pointed, upper profile linear to slightly convex, length 27.4–36.3% HL, about equal to length of orbit; length of orbit 29.0–32.0% HL; upper jaw extending anteriorly slightly beyond lower; first infraorbital pore large, length 3.6–5.0% HL (Fig. 2A); gill rakers 7–12 (Table 2), 0–1 on upper part of arch, 6–11 on lower part of arch, short and blunt; longest pectoral-fin ray 20.9–25.0% SL (sexually dimorphic; see below); third pelvic-fin ray 12.6–17.7% SL, longer than first and second; length of pelvic fin sexually dimorphic (see below); caudal peduncle compressed or roughly circular in cross section, length 14.7–18.3% SL, depth 3.1–4.1% SL. Skin covering elements of upper and lower

Table 1

Proportional morphometrics and meristics of *Triglops dorothy* n. sp. and of *T. pingeli* from the Sea of Okhotsk. Morphometric data are presented in percent of standard length (in mm). Statistical significance was tested by ANOVA when appropriate; ns = not significant.

	Triglops dorothy				*Triglops pingeli*			
	Holotype	*n*	Range	Mean ±SD	*n*	Range	Mean ±SD	Significance
Measurements								
Standard length (in mm)	155.0	21	68.5–155		27	88–184		
Snout length	9.0	20	7.9–9.6	8.9 ±0.5	27	6.8–10.1	9.1 ±0.7	
Orbit length	9.0	21	8.4–9.9	9.2 ±0.4	27	7.2–10.2	8.8 ±0.8	ns
Infraorbital pore width	2.2	12	0.7–2.3	1.3 ±0.4	27	0.5–1.9	1.1 ±0.4	$P<0.01$
Head length	31.0	21	28.4–32.3	29.9 ±0.9	27	26.4–33.6	30.5 ±1.6	
Head depth	12.3	19	11.3–13.5	12.5 ±0.5	27	11.0–14.5	12.5 ±0.8	
Interorbital width	3.0	21	2.7–3.5	3.1 ±0.2	27	2.1–3.4	2.7 ±0.4	$P=0.05$
Pectoral-fin ray length	22.6	21	20.9–25.0	22.8 ±1.2	27	20.5–29.6	23.8 ±2.4	ns
Pelvic-fin ray length	13.6	20	12.6–17.7	15.0 ±1.4	27	11.0–16.0	13.0 ±1.6	ns
Caudal-peduncle depth	3.7	21	3.1–4.1	3.7 ±0.2	27	2.6–4.3	3.5 ±0.3	ns
Caudal-peduncle length	15.5	21	14.7–18.3	16.8 ±1.0	27	14.7–21.1	16.8 ±1.7	ns
Counts								
Dorsolateral scales	31	21	18–31	25.0 ±3.3	27	28–37	32.9 ±2.9	$P<0.001$
Dermal folds	99	21	67–99	81.6 ±8.2	27	50–87	64.6 ±11.3	$P<0.001$
Breast scale rows	10	21	4–10	7.2 ±1.6	27	4–11	7.8 ±1.8	ns
Lateral-line scales	48	21	47–50	48.2 ±0.8	27	47–50	48.8 ±0.9	$P=0.02$
Dorsal-fin spines	11	21	10–11	10.9 ±0.3	27	10–11	10.7 ±0.4	
Dorsal-fin rays	24	21	23–25	24.1 ±0.6	27	23–27	25.0 ±0.9	ns
Anal-fin rays	24	21	23–25	23.8 ±0.5	27	22–25	23.7 ±0.7	ns
Pectoral-fin rays	18	21	17–19	18.1 ±0.5	27	18–21	19.3 ±0.8	$P<0.001$
Gill rakers	11	21	7–12	9.3 ±1.3	27	5–9	7.1 ±0.9	$P<0.001$
Vertebrae	12+34	21	11–12+33–35		4	12–13+32–36		

Table 2

Counts of dorsolateral scales, dermal folds, and gill rakers on the first arch of *Triglops dorothy* n. sp. and *Triglops pingeli* from the Sea of Okhotsk.

Dorsolateral scales

	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
T. dorothy	1	1		1	1	2	3	2	2	5		1	1							
T. pingeli											3	1	1	5	3	2		6	4	2

Dermal folds

	50	51	52	53	54	55	57	58	59	60	61	62	64	66	67	68	69	70	71	72	73	74	75	76	77	82	83	84	85	86	87	88	89	90	91	99
T. dorothy															1					2	1	2		1	2	1	2	1		2		1	1	1	2	1
T. pingeli	1	2	1	1	1	2	1	2			1	1	2	3	1				1			1	1		1		2		1							

Gill rakers

	5	6	7	8	9	10	11	12
T. dorothy			2	1	12	2	2	2
T. pingeli	1	5	13	7	1			

jaws, throat, ventral portion of cheeks, interopercle, extreme ventral tip of subopercle, and extreme dorsal margin of gill membrane naked; remaining part of head and dorsal half of body everywhere covered with rough, granular scales; approximately 10–16 close-set rows of tiny granular scales on anterior and dorsal periphery of eyeball, rows usually extending nearly to dorsalmost margin of eye. Oblique dermal folds terminating on caudal peduncle well before reaching ventral midline but sometimes crossing space between anus and base of anal fin. Dorsal-fin spines 10–11, anal-fin rays 23–25; vertebrae 45–47.

Color in preservation: dark brown dorsally, light brown, tan, to cream ventrally, with four dark saddles along dorsal length of body extending ventrally to, and sometimes slightly below, lateral line, anteriormost saddle situated beneath spinous dorsal fin, two beneath soft dorsal fin, posteriormost saddle on caudal peduncle; most specimens with a small saddle or narrow band of pigment crossing soft-dorsal fin at midlength (homologous to fifth saddle of many congeners). A series of dark, more-or-less equally spaced spots or streaks extending along length of body just ventral to lateral line, spots nearly always interconnected in males to form a conspicuous dark stripe across length of body, but not extending onto caudal-fin base. Anterior portion of lower lip darkly pigmented, sometimes interrupted at symphysis of jaw; upper lip with a dark streak across symphysis and two or three similar, more-or-less equally spaced streaks on each side, posteriormost streak contiguous with similar spot or streak on maxilla (pigmentation of lips more intense in males); males with a streak of pigment extending along dorsal margin of upper jaw from ventral margin of lacrimal to beneath and slightly past eye; a large, diffuse spot posterior to distal tip of maxilla (more heavily pigmented in males), sometimes forming an oblique streak extending from posterodorsal margin of maxilla posteroventrally to preopercle. Dorsomedial surface of opercle and posterodorsal margin of gill membrane densely covered with small melanophores, pigment extending ventrally along medial surface of branchiostegal rays (pigmentation often bleached away in old, poorly preserved material); lateral surface of distal portion of four or five posteriormost branchiostegal rays, as well as gill membrane in between, heavily peppered with small melanophores. Each spine and ray of dorsal fin (but not membrane in between) with two to four, dark, more-or-less equally spaced spots. Four to seven narrow, more-or-less equally spaced bands of dark pigment across dorsalmost or nonexerted rays of pectoral fin; a small, dark spot at dorsal insertion of pectoral fin often continuous with pigment extending ventrally to mid-base of fin. Anal fin usually unpigmented, but some specimens with rays darkly pigmented or lined with rows of melanophores, pigmentation especially intense in males; pelvic fins usually unpigmented, but some males with distal tips of first and second rays lightly pigmented. Base of caudal fin with dark dorsal and ventral spots, ventral spot usually more distinct than dorsal; males with a dark spot near distal end of dorsal and ventral lobes of caudal fin, sometimes faint and difficult to discern in preserved specimens.

Sexual dimorphism

Females with pectoral-fin rays slightly shorter (22.6–23.4% SL) than those of males (20.9–25.0% SL). Females with pelvic-fin rays slightly shorter (12.6–14.7% SL) than those of males (12.7–17.7% SL); tissue surrounding pelvic-fin spine and first ray expanded in males.

Statistical analyses

Univariate analyses of morphometric characters indicated that only interorbital width and the length of the first infraorbital pore exhibited significant differences between *T. dorothy* and *T. pingeli* (Table 1). The analyses of meristic characters, on the other hand, revealed significant differences in several characters, including fewer dorsolateral scales, lateral-line scales, and pectoral-fin rays, and a greater number of dermal folds and gill rakers in *T. dorothy* than in *T. pingeli* (Table 1). Three-way plots of dorsolateral scales, dermal folds, gill rakers, and pectoral-fin rays exhibit a clear separation between *T. pingeli* and *T. dorothy* (Fig. 2B).

In plots of scores from the PCA of meristic characters, two distinct clusters, one for each of the two species, were evident (Fig. 3A). The primary axis of separation was PC1 (30.2% of total variance), which was heavily loaded positively on dorsolateral scales and gill rakers and negatively on lateral-line scales; loadings along the PC2 axis (22.4% of total variance) were heavily loaded positively for breast-scale rows and negatively for anal-fin rays and dorsal-fin rays (Table 3). In contrast, broad overlap was displayed in the analysis of morphometric characters, with individuals of *T. dorothy* clustering among the larger group of *T. pingeli* from the Sea of Okhotsk (Fig. 3B). The first principle component (PC) was interpreted as a size component accounting for 95.5% of the total variance. In plots of the scores of the sheared second and third shape components (1.5% and 1.1% of total variance, respectively), the greatest dispersion was along the PC2 axis (Fig. 3B, C), and loadings along this axis were highest for interorbital width, snout length, and caudal-peduncle depth (Table 3). Loadings along the PC3 axis were highest for pectoral-fin ray length, ventral caudal-peduncle length, and orbit length (Table 3).

The single linear discriminant function equation produced was highly significant (χ^2=96.5, 6 df, P<0.0001):

$$D = 0.894(ds) - 0.691(g) + 0.530(p) - 0.508(df) - 0.284(io) + 0.098(ll),$$

where D = the discriminant score of an individual;
ds = number of dorsolateral scales;
g = number of gill rakers;

Table 3

Factor loadings for standard principal component (PC) analysis of meristic characters and sheared PC analysis of morphometric characters for *Triglops dorothy* n. sp. and for *T. pingeli* from the Sea of Okhotsk.

	PC1	PC2	PC3
Meristics			
Dorsal-fin rays	−0.3678	−0.4269	0.1352
Lateral-line scales	−0.3886	−0.1695	0.2437
Anal-fin rays	−0.2007	−0.5078	0.3611
Dermal folds	−0.4517	−0.0691	0.4440
Pectoral-fin rays	−0.3750	0.3030	0.3137
Dorsolateral scales	−0.3668	0.3919	0.2438
Breast scale rows	0.0861	0.4464	0.4980
Dorsal-fin spines	0.1918	0.1278	0.0765
Gill rakers	0.3875	−0.2560	0.4281
Morphometrics			
Standard length	0.3446	0.0348	0.0764
Snout length	0.3541	0.2603	−0.2662
Orbit length	0.3117	0.2043	−0.4521
Head length	0.3547	0.15793	−0.1867
Head depth	0.3340	0.0861	−0.2887
Interorbital width	0.2999	−0.7286	−0.0446
Pectoral-fin ray length	0.3431	0.2007	0.5844
Caudal-peduncle depth	0.3266	−0.5013	0.0598
Caudal-peduncle length	0.3271	0.1857	0.5036

p = number of pectoral-fin rays;
df = number of dermal folds;
io = interorbital width divided by SL; and
ll = number of lateral-line scales.

All individuals with negative scores had been previously identified as *T. dorothy*, whereas those with positive scores were *T. pingeli*. The discriminant function equation correctly classified all individuals, as did the cross-validation procedure.

Distribution and habitat

Triglops dorothy is known only from the southern Sea of Okhotsk: 19 specimens collected in or adjacent to Aniva Bay, at the southern end of Sakhalin Island, Russia; and two specimens from north of Kitami, Hokkaido (Fig. 4). The material was trawled off bottoms of mud, fine sand, rock, and clay, at depths of 73–117 m.

Etymology

The species is named in honor of Dorothy Thomlinson Gilbert, great granddaughter-in-law of the eminent ichthyologist and fisheries biologist Charles Henry Gilbert, for her generous and steadfast support to graduate students in ichthyology at the University of Washington, Seattle, in establishing the William W. and Dorothy T. Gilbert Ichthyology Research Fund. The specific epithet is to be treated as a noun in apposition.

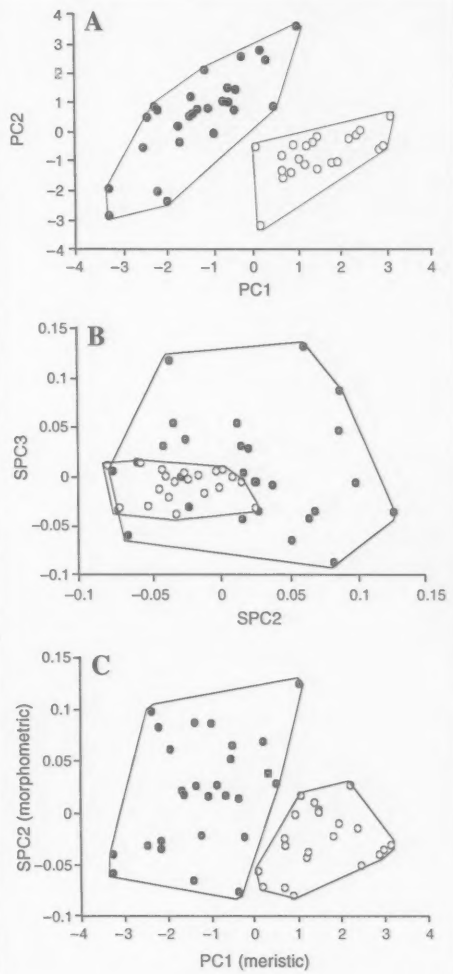

Figure 3

Plots of sheared principal component (SPC) and standard principal component (PC) scores for morphometric and meristic characters of *Triglops dorothy* n. sp. (•) and *T. pingeli* (○): (**A**) meristic characters only; (**B**) morphometric (SPC2 and SPC3) characters only; (**C**) morphometric (SPC2) and meristic characters (PC1).

Discussion

Credit for the discovery of *T. dorothy* is given to Charles Henry Gilbert (Pietsch, 1993; for biographical information on Gilbert, see Dunn, 1997), who, soon after the material was collected, recognized its uniqueness.

Although the USNM catalog ledger has "cotype" handwritten next to each catalog number (all 19 CAS-SU and USNM specimens are labeled as such) and Smithsonian Institution accession file no. 54484, dated 6 September 1912, lists the material as "co-types described by Dr. Gilbert" (Palmer[1]), a description was never published.

Why Gilbert, on closer inspection, chose not to formally recognize *T. dorothy* is probably because of its extreme similarity to *T. pingeli*. *Triglops dorothy* is easily confused with the latter species, but can be distinguished satisfactorily on the basis of only three meristic characters that overlap slightly between the two species in the Sea of Okhotsk (Tables 1–2): the number of oblique, scaled dermal folds below the lateral line (67–99 in *T. dorothy* versus 50–87 in *T. pingeli*), the number of dorsolateral scales (18–31 in *T. dorothy* versus 28–37 in *T. pingeli*), and the number of gill rakers (7–12 in *T. dorothy* versus 5–9 in *T. pingeli*). A significant difference in the length of the first infraorbital pore (Fig. 2A) reflects differences in the overall shape of the snout of the two species. In *T. dorothy*, the snout is slightly shorter and slightly more ventrally oriented than in *T. pingeli*.

Triglops dorothy is known only from the shallow continental shelf of the southern Sea of Okhotsk between Sakhalin Island and Hokkaido, an area that is thought to have served as a refugia for other species during the Quaternary glacial maximum (see Noll et al., 2001; Brykov et al., 2003). During this period, glaciers extended to just north of Terpeniya Bay on Sakhalin Island and to central Iturup Island in the southern Kurils, leaving the southern and central part of the Sea of Okhotsk ice-free. This region is thought to have been relatively isolated during this time from the Pacific Ocean and the Sea of Japan because of lower sea levels that connected the southern coastlines from Sakhalin to Hokkaido and the southern Kuril Islands (Kryvolutskaya, 1973; Pietsch et al., 2001; 2003). These land connections may have served to split and isolate populations of benthic marine fishes, such as *T. pingeli*, providing the opportunity for allopatric speciation of *T. dorothy*.

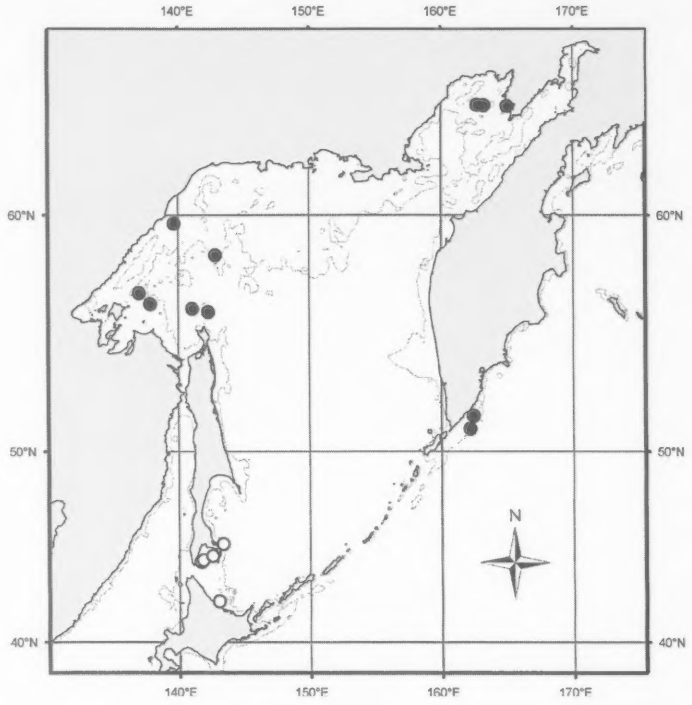

Figure 4

Distribution of *Triglops dorothy* n. sp. (○) and *T. pingeli* (●) in the Sea of Okhotsk based on material examined. Each symbol may represent more than one capture.

Acknowledgments

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Appendix—Comparative material examined

Triglops pingeli: HUMZ 54593 (female, 164 mm), HUMZ 54594 (female, 180 mm), HUMZ 54674 (male, 158 mm), HUMZ 55266 (female, 184 mm), HUMZ 55267 (female, 165 mm), HUMZ 55268 (female, 176 mm), West Kamchatka, 59°00′N, 168°00′E, 80 m, 5 June 1976; HUMZ 55052 (female, 170 mm), HUMZ 55055 (female, 171 mm), HUMZ 55058 (female, 167 mm), HUMZ 55062 (male, 154 mm), HUMZ 55089 (male, 150 mm), East Kamchatka, 51°25′N, 158°04′E, 135–140 m, 21 May 1976; HUMZ 55154 (female, 140 mm), Penjinskii Bay, 59°59′N, 160°00′E, 112–113 m, 6 June 1976; HUMZ 56400 (female, 104 mm), East Kamchatka, 50°58′N, 157°52′E, 242–245 m, 22 May 1976; HUMZ 56466 (female, 127 mm), Gizhiga, 61°01′N, 158°17′E, 94–95 m, 7 June 1976; HUMZ 57984 (female, 120 mm), Sea of Okhotsk, 56°40′N, 143°22′E, 310 m, 22 September 1976; HUMZ 58051 (male, 114 mm), HUMZ 60855 (female, 119 mm), Sea of Okhotsk, 55°30′N, 139°00′E, 117 m, 7 September 1976; HUMZ 60702 (male, 123 mm), Sea of Okhotsk, 57°38′N, 141°00′E, 121 m, 14 October 1976; HUMZ 60799 (female, 121 mm), Sea of Okhotsk, 57°38′N, 141°00′E, 121 m, 14 October 1976; HUMZ 60808 (female, 140 mm), HUMZ 60810 (female, 126 mm), HUMZ 60811 (female, 124 mm), Sea of Okhotsk, 54°54′N, 142°57′E, 110 m, 6 September 1976; HUMZ 60919 (female, 88 mm), Gizhiga, 61°00′N, 158°41′E, 88–90 m, 7 June 1976; HUMZ 60941 (female, 125 mm), HUMZ 60952 (female, 125 mm), Sea of Okhotsk, 55°00′N, 142°02′E, 121 m, 6 September 1976; HUMZ 61063 (male, 120 mm), HUMZ 61068 (female, 128 mm), Sea of Okhotsk, 55°09′N, 139°38′E, 124 m, 7 September 1976.

A comparison of two fishery-independent survey programs used to define the population structure of American lobster (*Homarus americanus*) in the Gulf of Maine

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Abstract—The population structure and abundance of the American lobster (*Homarus americanus*) stock in the Gulf of Maine are defined by data derived from a fishery-independent trawl survey program conducted by the National Marine Fisheries Service (NMFS). Few sampling stations in the survey area are located inshore, in particular along coastal Maine. According to statistics, however, more than two thirds of the lobster landings come from inshore waters within three miles off the coast of Maine. In order to include an inshore survey program, complementary to the NMFS survey, the Maine Department of Marine Resources (DMR) initialized an inshore survey program in 2000. The survey was modeled on the NMFS survey program, making these two survey programs comparable. Using data from both survey programs, we evaluated the population structure of the American lobster in the Gulf of Maine. Our findings indicate that lobsters in the Gulf of Maine tend to have a size-dependent inshore-offshore distribution; smaller lobsters are more likely to stay inshore and larger lobsters are more likely to stay offshore. The DMR inshore and NMFS survey programs focused on different areas in the Gulf of Maine and likely targeted different segments of the stock. We suggest that data from both survey programs be used to assess the lobster stock and to describe the dynamics of the stock in the Gulf of Maine.

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Many fish species experience size-dependent inshore-offshore movement at a certain stage (or stages) of their lives, resulting in size-dependent distribution. Shallow inshore waters along coasts in many parts of the world are primary nursery or spawning grounds (or both) for many commercially and recreationally important fish species (Taylor, 1953; Caddy, 1975). Juveniles of many fish species inhabit shallow inshore waters but migrate to deeper waters with increasing size (Chen et al., 1997). This size-dependent movement can also be observed in other life history stages such as when mature fish migrate to spawning grounds (Rowling, 1994). One consequence of such a size-dependent movement is that spatial distribution of fish becomes size-specific. Many ocean fish aggregate in their habitats according to size (Paloheimo and Dickie, 1964; Caddy, 1975; Hilborn and Walters, 1992).

The American lobster (*Homarus americanus*) is distributed throughout the northwest Atlantic from the Straight of Belle Isle, Newfoundland, to Cape Hatteras, North Carolina. It supports the most valuable commercial fishery in the Northeast United States (ASMFC, 2000). This lobster is most abundant in shallow coastal waters but can also be found in water depths of 700 meters (Cooper and Uzmann, 1980; Lawton and Lavalli, 1995). Various surveys and studies have indicated that there are large differences in lobster density among different areas and different water depths (Wahle and Steneck, 1992; Wilson, 1998; Palma et al., 1999).

Lobsters tend to move little during their first year on the bottom (Wahle, 1992; Palma et al., 1999). The daily and annual movement range increases with their sizes. After they reach harvestable size, their annual range of movement is about 2 to 3 kilometers (Krouse, 1980). Sublegal-size lobsters generally have small seasonal movements (<1 km/year) and are largely found near shore. Larger lobsters are more mobile (<20 km/year) and show progressively offshore deep

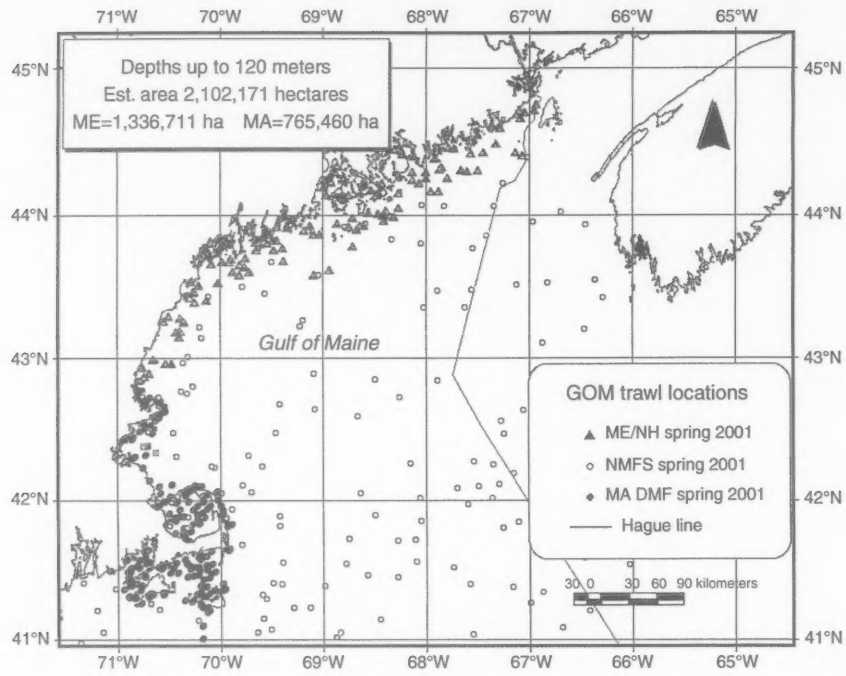

Figure 1

Geographical areas of the Maine Department of Marine Resources (DMF) inshore survey program and National Marine Fisheries Services NMFS survey program. ME/NH=Maine and New Hampshire; MA DMF=Massachusetts Department of Marine Fisheries.

movements in the fall, returning to inshore shallow areas in the spring.

The range of movement increases further after they reach maturity. However, there are large differences in migratory behaviors among individuals (Pezzack and Duggan, 1986). In general, mature legal-size lobsters are more abundant offshore and in deeper waters (Harding and Trites, 1989). Large egg-bearing lobsters have been shown to exhibit long migrations (>100 km) (Krouse, 1980; Campbell, 1983).

Size-dependent inshore-offshore distributions have been reported in many lobster studies (Skud, 1969; Cooper et al., 1975; Briggs, 1985; Campbell and Pezzack, 1986; Cobb et al., 1989; Harding and Trites, 1989). Information collected in commercial fisheries and scientific surveys has revealed that the spatial distribution of the American lobster may be size-dependent and that lobster of large sizes are more likely found in deep waters. This pattern has been identified but not quantified (Wilson, 1998). The spatial distribution of lobsters varies with seasons and scales with the size of lobsters.

The distribution and abundance of lobsters have been studied with a variety of techniques. Diver and submarine and ROV surveys are generally limited to nearshore areas or in temporal resolution. Tagging programs are often limited in area and time covered and in number of lobsters tagged and returned. A fisheries-dependent sea sampling program covers a limited number of fishing boats within limited areas and thus may not be able to provide an overall picture of lobster distribution and abundance within the lobster stock area in the Gulf of Maine. Until recently, the only comprehensive fishery-independent data were those obtained from the NMFS Gulf of Maine fall and spring trawl surveys. NMFS has conducted a randomized stratified survey since the mid 1960s, but this survey is limited to depths deeper than 50 meters. In fact, the majority of the sampling stations are located in waters deeper than 120 meters as a result of an increased proportion of untrawlable areas and problems with lobster gear in inshore waters. In the fall of 2000, the DMR began a coast-wide inshore trawl survey, which mainly covered waters shallower than 120 meters (Fig. 1).

The spatial distribution of lobsters is largely restricted to the nearshore areas. Although found throughout the Gulf of Maine, 80% of the landings are estimated to come within three miles from shore (ASMFC, 2000). Clearly the lobster fishery will follow the concentrations of lobsters, although seasonal changes in movements and trapability of lobsters may make less productive fishing areas desirable to limited portions of the fleet at certain times of the year. As the fishing effort for lobster has increased, the traditional inshore fishery

has expanded to nearshore waters (from 4.8 km to 32.2 km from shore). There is also a deepwater fishery for lobster that occurs farther from shore.

An optimal and effective fisheries management plan requires a high-quality stock assessment, which, in turn, is dependent on data collected from fisheries-dependent and fisheries-independent survey programs. One of the most important pieces of information used in a lobster stock assessment is the abundance index which is derived from the fisheries-independent bottom trawl surveys conducted by NMFS. Because trawling is difficult in coastal waters, few sampling stations in the NMFS survey are located inshore, specifically along coastal Maine from which the majority of the lobster landings are landed. Previous studies have also indicated that inshore habitats are critical to the lobster fishery. Thus, it is critical to develop an inshore survey program that can cover the waters off coastal Maine. The data collected from such a program can overcome the problem of a lack of inshore coverage in the NMFS survey. An inshore survey and the NMFS survey can complement each other for a more complete abundance index of the lobster stock in the Gulf of Maine.

In order to identify the importance of the DMR inshore survey, we compared the differences between the NMFS survey and DMR inshore survey in 1) the size composition of survey catches and their temporal trends; 2) temporal trends in abundance indices; and 3) average size and weight of lobsters. From our comparative study, we determined whether the lobster stock in the Gulf of Maine has a size-dependent inshore-offshore distribution and whether it is essential to include both sampling programs in a stock assessment to adequately describe the population dynamics of lobsters in the Gulf of Maine.

Methods and materials

Like the NMFS surveys, the inshore trawl survey is conducted during the spring and fall of each year. It has a stratified random design modeled after the NMFS and Massachusetts Department of Marine Fisheries (MADMF) surveys. The design has four depth strata (9–37 m, 37–64 m, 64–100 m, >100 m [its outer boundary roughly delineated by the 12-mile or 22-km limit]) and five regions based on oceanographic, geologic, and biological features. The fourth stratum was added in the spring of 2003. It expands the coverage area to equal the area in federal waters covered by the Atlantic States Marine Fisheries Commission (ASMFC) and allows some overlaps between annual inshore trawl survey area and the NMFS survey area. It also slightly reduces the sampling pressure in the shallower strata, which has been of concern to fixed-gear fishermen in the past. To randomize the survey area (~13,720 km^2), each depth stratum is divided into 1-nmi (i.e., 1.852 km) sampling grids. A target of 100 stations is selected for sampling in each survey, resulting in a sampling density of about 1 station /137 km^2. This density compares to the sampling density of the NMFS survey (1 station/892 km^2) and Massachusetts' survey (1 station/65 km^2). The number of stations per stratum is allocated in proportion to the area of each stratum. When a station is encountered that cannot be towed, an alternate tow is selected nearby over similar depth.

Trawl design considerations for the survey included effectiveness of the gear for sampling the complex bottom in the Gulf of Maine and approximate comparability with previous and ongoing surveys, such as that performed by the NMFS. Net tapers are cut to permit the shape of the net to be of maximum height, while allowing the net to remain tight on the bottom. The net is shackled from the footrope to the frame by using two 0.95-cm shackles on a banded wire that runs parallel with the footrope. Heavy rubber wing bobbins retard bottom wing lift. The top leg is constructed of 5.1-cm mesh overall and has a 1.3-cm mesh liner in the codend. Doors are 7.5 Bison doors. Attached to the 21.3-m-long, 1.59-wide footrope is a roller frame. The 3.05-m-wide bosom section is made up of 20.3-cm rubber disks on 15.2-cm centers, and there are eight evenly spaced toggles. The spacing is maintained by small 10.2-cm cookies strung between the disks. Chain sweeps were not used. The headrope is 17.4 meter in length.

Environmental data, including temperature and salinity profiles, wind, sea state, and weather, were collected at each station. A standard trawl tow, 20 minutes in duration, was made at each station. Shorter tow times were accepted under certain circumstances, such as the presence of fixed gears or untrawlable bottom in the survey pathway. Tow speed was maintained at 2.1 to 2.3 knots (i.e., 3.9 km/h to 4.3 km/h) and tow direction was oriented toward the tidal current whenever possible. All sampling was conducted during the day. After each tow, the net was brought aboard and emptied onto a sorting table. All individuals were identified and sorted by species. All lobsters were immediately separated and processed while the rest of the catch was sorted. Total weights (by sex), carapace length (mm), shell condition, presence and stage of eggs, V-notch condition, and injuries to the lobsters caused by the trawl were recorded. All lobsters were measured and the data were recorded in electronic format for analysis and made available on compact disk (CD). The data were geo-referenced and incorporated into geographic information systems (GIS) for analysis.

A no. 36 Yankee bottom trawl has generally been used in NMFS bottom trawl surveys. The trawl net is towed at approximately 3.5 knots (i.e., 6.5 km/h) for 30 minutes at each station. The survey is based on a stratified random design. Strata are defined according to water depth, latitude, and historical fishing patterns. Within each stratum, stations are assigned randomly; the number of stations allotted to a stratum is in proportion to its area (approximately one station per 892 km^2). Specifications for NEFSC standard no. 36 Yankee bottom trawl are defined by the NMFS.[1]

[1] NMFS (National Marine Fisheries Service). Website: http://www.nefsc.noaa.gov/femad/ecosurvey/mainpage. [Accessed August 2004.]

The trawl used in the DMR survey is similar in design, but smaller than that used in NMFS, although the size of the mesh liner in the codend is the same. The DMR survey trawl net is also towed for less time (20 minutes versus 30 minutes for NMFS).

The data collected in the inshore survey were analyzed according to the methods described below. The stratified mean number of lobsters per tow, $\bar{X}$, can be calculated as

$$\bar{X} = \frac{1}{A}\sum_{k=1}^{g} A_k \bar{X}_k,$$

where g = the number of stratum in the survey;
A_k = the survey area in stratum k;
A = the total area covered by the survey ($A=\sum_{k=1}^{g} A_k$); and
$\bar{X}_k$ = the average catch per tow in stratum k.

The $\bar{X}_k$ can be calculated as

$$\bar{X}_k = \frac{1}{T_k}\sum_{t=1}^{T_k} X_{k,t},$$

where $X_{k,t}$ = catch in tow t, stratum k; and
T_k = the total number of tow in stratum K.

The variance for the stratified mean, $S^2(\bar{X})$, can be estimated as

$$S^2(\bar{X}) = \frac{1}{A^2}\sum_{k=1}^{g} A_k^2 S^2(\bar{X}_k),$$

where $S_2(\bar{X}_k)$ = the variance for the mean number per tow in stratum k, which can be estimated as

$$S^2(\bar{X}_k) = \frac{\sum_{t=1}^{T_k}(X_{k,t} - \bar{X}_k)^2}{n_k(n_k - 1)}.$$

The mean length of the lobster population is estimated as

$$\bar{L} = \frac{\sum_{k=1}^{g} A_k \bar{C}_k}{\sum_{k=1}^{g} A_k \bar{X}_k}.$$

The average length fish per tow, $\bar{C}_k$, in the above equation is calculated as

$$\bar{C}_k = \frac{\sum_{t=1}^{T_k}\sum_{j=1}^{200} L_j X_{j,t,k}}{T_k},$$

where L_j = the lobster carapace size ranging from 1 to 200; and
$X_{j,t,k}$ = the number of the lobsters in size j, tow t, and stratum k.

The stratified mean number of lobsters of length j per tow, $\bar{X}_j$, can be estimated as

$$\bar{X}_j = \frac{1}{A}\sum_{k=1}^{g} A_k \bar{X}_{j,k},$$

where the mean number of lobsters of size j per tow, $\bar{X}_{j,k}$, is estimated as

$$\bar{X}_{j,k} = \sum_{t=1}^{T_k} \frac{X_{j,t,k}}{T_k}.$$

The above equations were used for both the original data (without any transformation) and the data transformed by using log(x+1). For results derived from the log (x+1) transformed data, a retransformation was done to retransform the log-based results to the results of the original scale by using the following equation:

$$U = \exp(Y + \sigma_U^2/2),$$

where Y = the statistics derived from log(x+1) transformed data;
U = is the re-transformed statistics; and
σ_U^2 = the population variance estimates as

$$\sigma_U^2 = \frac{1}{\sum_{k=1}^{T_k} A_k}\sum_{k=1}^{T_k}\left[A_k S_{Y_k}^2 + A_k\left(\bar{Y}_k - \frac{\sum_{k=1}^{T_k} A_k \bar{Y}_k}{\sum_{k=1}^{T_k} A_k}\right)^2\right],$$

where $S_{Y_k}^2$ and $\bar{Y}_k$ = the variance and mean value of log(x+1) transformed data in stratum k, respectively.

Using the above equations, we estimated the stratified mean number of lobsters per tow for original and log(x+1) transformed data, the stratified mean size frequency, and the stratified mean size of lobsters for the Maine inshore surveys. The average weight of lobsters for each tow was also calculated and then plotted against the average depth of the tow to identify if there was any size-depth dependent distribution. It should be noted that observations of zero lobsters occurred only for a small number of tows in the years covered in our study. Thus for the DMR inshore survey, there was no need to use methods such as delta transformation, which is often used in dealing with zero-inflated survey data with a large number of zero lobster catches.

The stratified mean number of lobsters per tow, stratified mean size frequency, and stratified mean size of lobsters for the NMFS survey program were obtained from the NMFS (Idoine[2]). Because of the large number

[2] Idoine, J. 2004. Personal commun. Northeast Fisheries Science Center, 166 Water Street, Woods Hole, MA 02543-1026.

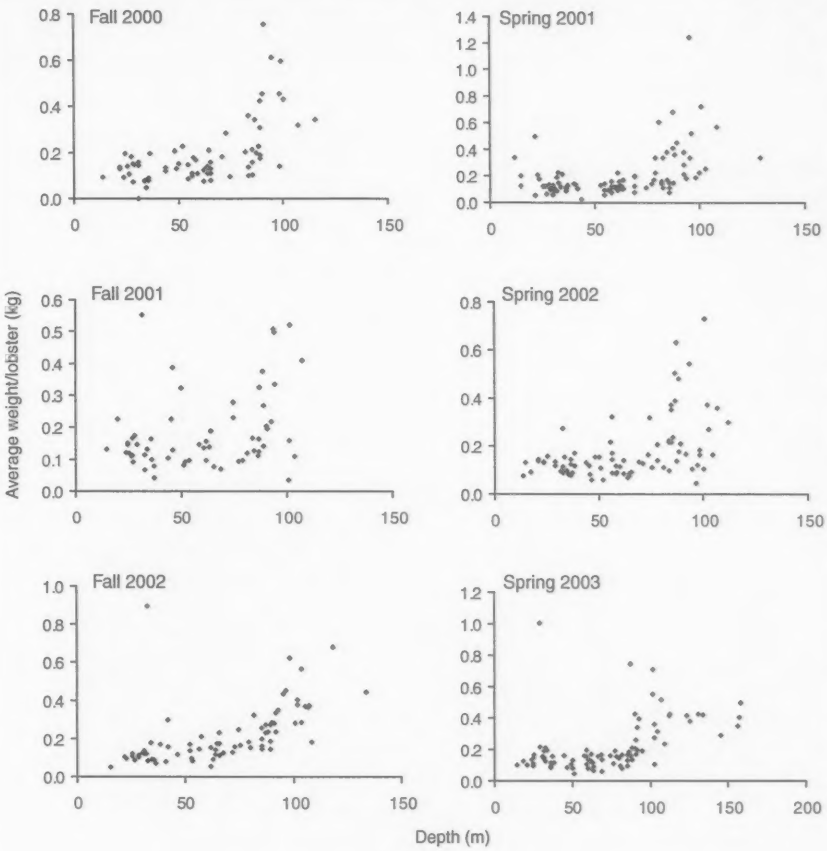

Figure 2

The changes in the average weight of American lobster (*Homarus americanus*) with the average depth of survey for each tow in each sampling season and year.

of tows with zero lobsters in the NMFS survey, a delta transformation was used to estimate the stratified mean of lobsters per tow. Because the DMR inshore survey program began in fall 2000, we used data from the NMFS survey program for the same time period only for our comparison of data. Currently the stock assessment uses only female data because females and males exhibit similar pattern; therefore we report results for females only in the present study.

Results

The average weight per lobster tended to be low and relatively constant for lobsters caught in the tows conducted in waters shallower than 73 m but increased greatly with increased depths (Fig. 2). The average carapace length for the first two depth strata, which were shallower than 72 m, were similar for each year and sampling season, but were much smaller than the average carapace length of lobsters in the third depth stratum which was deeper than 73 m (Table 1). For example, for the 2000 fall survey, the average lobster size for depth strata I and II were 61.9 and 64.4 mm—much smaller than 80 mm for stratum III. These distributional patterns in the lobster average weight and average size were consistent among years and seasons, indicating that lobsters inhabiting waters shallower than 73 m were mainly small individuals, whereas lobsters in waters deeper than 73 m were much larger than those in the shallow waters. A fourth depth stratum was used in the 2003 spring survey, where the average towing depth was 125 m. The average size of lobsters sampled in this stratum was 99.5 mm, which was similar to the stratified mean size of lobsters estimated in the NMFS survey program (Fig. 3).

There were no significant differences in size distributions among years in a given sampling season (i.e.,

Table 1

Summary of the mean carapace length of lobsters (*Homarus americanus*) and average survey depth for different depth strata in the inshore surveys. Roman numerals stand for depth strata: I=9–37 m; II=37–64 m; III=64–100 m; IV=>100 m). na=not available.

		Mean carapace length (mm)				Average depth (m)			
Year	Season	I	II	III	IV	I	II	III	IV
2000	fall	61.9	64.4	80.0	na	28.6	57.9	89.2	na
2001	fall	63.6	63.0	78.0	na	28.2	53.7	88.8	na
2002	fall	63.6	69.9	84.4	na	29.8	57.1	93.0	na
2001	spring	66.1	63.7	79.9	na	29.5	59.2	89.0	na
2002	spring	61.5	61.1	78.6	na	29.7	56.4	89.7	na
2003	spring	68.6	64.0	74.9	99.5	29.3	59.0	87.7	125.4

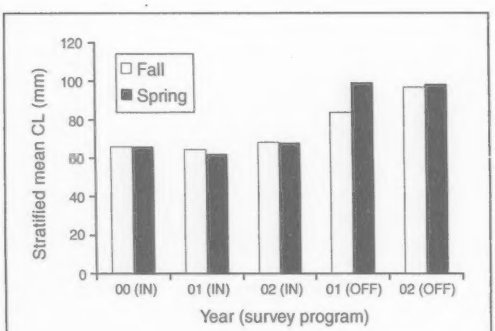

Figure 3

The stratified mean carapace length (CL) for American lobsters (*Homarus americanus*) for each sampling season and year for the Maine Department of Marine Resources inshore (IN) and the National Marine Fisheries Service offshore (OFF) survey programs. 00=2000; 01=2001; 02=2002.

either fall or spring survey) for the inshore survey (Fig. 4). For the NMFS survey, however, significant differences were found in size distributions between 2001 and 2002 in the fall surveys, but not between the two years in the spring surveys. The seasonal differences (i.e., spring vs. fall) in size distribution within a year were much larger than the between-year differences for the inshore fall or spring survey. For the NMFS survey, however, the seasonal differences in size distributions were large in 2001, but small in 2002 (Fig. 4). The large seasonal differences in a year compared to the between-year differences in a season in size distributions for the inshore survey indicate that seasonal factors are important in determining the size distribution of lobsters in the inshore waters. Such seasonal variability was not so clear for the offshore waters.

Large differences in size distributions were observed between the DMR inshore and NMFS surveys; the NMFS surveys consisted of lobsters of much larger sizes than those sampled inshore (Fig. 4). This finding may indicate that the inshore survey program had a limited coverage of the lobsters of large sizes, whereas the NMFS survey program had a limited coverage of the lobsters of small sizes. The size composition of lobsters from the NMFS survey had large variations. This result was probably due to the small number of lobsters caught in the NMFS survey. An increase in sample size could make the size composition curve smoother, and thus better defined. Compared with size composition of Amercan lobsters from the NMFS survey, size composition for the inshore survey was better defined, probably as a result of the larger number of lobsters caught in the inshore survey.

The stratified mean sizes of lobsters were similar among years for the same sampling season in the inshore survey (Fig. 3). For 2001, the stratified mean size in the fall survey was almost the same as that in the spring survey. For 2002, however, the stratified mean size in the fall survey was about 7 mm larger than that in the spring survey (Fig. 3). The stratified mean size of the 2001 fall survey was about 16 mm smaller than that of the 2001 spring survey (Fig. 3). The stratified mean size of lobsters in the NMFS survey was much larger than that of the inshore survey. This reconfirms the results derived from the comparisons of size distributions between the inshore and NMFS surveys (Fig. 4).

The abundance index derived from the inshore survey program revealed a consistent temporal pattern with original data or retransformed data (Table 2). For the original data and retransformed data, the fall inshore survey abundance index was the highest in 2001, followed closely by 2002, and the abundance index in 2000 was the lowest. For the log-transformed data, however, the differences among the three years were small (Table 2). For the spring survey, the abundance index in 2002 was much higher than the abundance indices in 2001 and 2003, and the abundance indices in 2001 and 2003 were similar (Table 2). The delta mean abundance indices of the NMFS survey program were higher in 2002

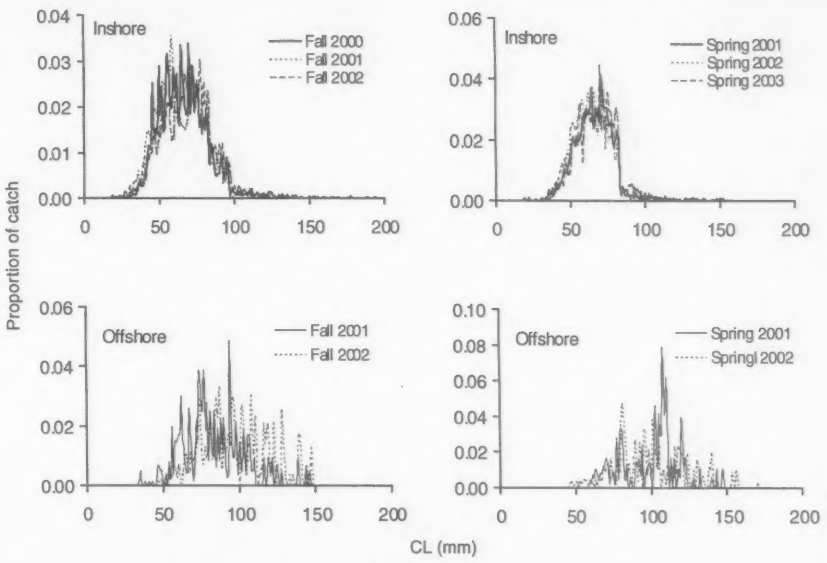

Figure 4

The size (of carapace [CL]) distributions for American lobsters (*Homarus americanus*) for each sampling season and year for the Maine Department of Marine Resources inshore and the National Marine Fisheries Service offshore survey programs.

Table 2

Estimates of the survey abundance index obtained from the original data and log(x+1) transformed data, and the retransformed abundance index derived from log(x+1) transformed data. "Lower B" and "upper B" are the lower and upper boundaries of the 95% confidence intervals.

		Statistics		Fall		Spring	
Index		2000	2001	2002	2001	2002	2003
Original (inshore)	Mean	55.5	68.5	64.1	22.6	49.2	21.2
	Lower B	32.7	45.1	51.7	16.4	34.7	15.8
	Upper B	78.2	91.9	76.5	28.8	63.6	26.7
Log(x+1)	Mean	2.9	2.9	3.1	2.1	2.7	2.2
transformed (inshore)	Lower B	2.6	2.5	2.8	1.9	2.4	1.8
	Upper B	3.2	3.3	3.5	2.4	3.0	2.6
Retransformed (inshore)	Mean	54.5	119.6	88.8	25.1	62.4	24.3
	Lower B	39.5	81.5	62.4	19.6	45.9	16.1
	Upper B	75.2	175.1	126.3	32.0	84.7	36.5
Delta mean (NMFS)	Mean		1.52	2.67	1.63	2.53	

than those in 2001 for both the fall and spring surveys. Thus, for fall, DMR inshore survey program had a different temporal pattern between 2001 and 2002, compared with the NMFS surveys, whereas for spring, the temporal pattern of 2001 and 2002 was same for the two sampling programs.

Discussions

Large differences were found in size compositions and mean sizes of lobsters between the DMR inshore and NMFS surveys. Such differences indicated that large lobsters are more likely to appear in the NMFS survey,

and small lobsters are more likely to appear in the inshore survey. This is true for both sampling seasons, fall and spring. Two possible hypotheses can be developed to explain such patterns: one is that large differences in size compositions exist in lobsters inhabiting the areas covered by the inshore and NMFS survey programs, and the other is that the observed differences in size compositions between the DMR inshore and NMFS surveys result from differences in gear selectivity for lobsters of different sizes. The first hypothesis implies that lobsters have size-dependent inshore-offshore distribution, namely that large lobsters tend to inhabit deep waters and small lobsters inhabit shallow waters. The second hypothesis implies that although lobsters may have no size-dependent inshore-offshore distribution, the NMFS survey gears are selective for large lobsters and the inshore survey gears are selective for small lobsters in the population. Such a difference in gear selectivity between the two programs may result from differences between the two sampling programs not only in sampling gears but in towing speed and duration.

In order to determine which hypothesis is more plausible, we evaluated size composition for a depth stratum covered by both the DMR inshore and NMFS surveys. In the 2003 spring inshore survey, the fourth depth stratum had an average depth of 125 m which overlapped depth ranges in the NMFS surveys. The average size of lobsters in the fourth depth stratum in the 2003 spring inshore survey was almost identical to the average size of lobsters in the NMFS survey (Table 1 and Fig. 3). This finding indicates that the inshore survey gears can, like the NMFS survey gears, catch large lobsters if they are present in the areas covered by the survey program. Likewise, the differences in the average size between the first three strata and the fourth stratum (Table 1) in the 2003 spring inshore survey are likely to result from a lack of large lobsters in the first three strata in the inshore surveys, rather than from gear selectivity. Thus, the differences in size composition of lobsters between the DMR and NMFS surveys are likely the result of the differences in the size composition of the lobster population between the areas covered by the inshore and NMFS surveys, rather than the result of sampling methods. Although our study could not exclude the impacts of possible differences in gear selectivity on the survey size compositions, the results of our study seem to support the first hypothesis that the lobsters have a size-dependent inshore-offshore distribution, where large lobsters are more likely to be found in deep waters and small lobsters are to be found in shallow inshore waters. Thus, the two sampling programs tend to cover different segments of the stock in the Gulf of Maine. In order to have an adequate representation of the lobster population, it is necessary to include data from both sampling programs to describe the lobster population dynamics in the Gulf of Maine.

The temporal changes in the abundance index in the spring surveys were rather consistent between the DMR and NMFS surveys and for data on the original and log scales. However, for the fall survey, the delta mean of the NMFS survey was consistent only with the mean of log-transformed data, but not with the mean of the original data and the mean retransformed data from the mean of log-transformed data (Table 2). This finding raises an interesting question regarding the type of data transformation we should choose and the potential impacts of each type on detecting temporal changes in the population abundance. A different choice of data transformation methods may lead to different interpretations of temporal variations in stock abundance.

If the size-dependent inshore-offshore distribution pattern is not taken into consideration in making stock assessments, the entire stock will not be managed. We may need a separate set of abundance indices and size compositions for the lobsters in the inshore and offshore waters to describe the population dynamics of lobsters of different size groups more accurately. The inclusion of only NMFS survey data or inshore survey data in the stock assessment may lead to errors in determining the status of the lobsters if the population dynamics of large lobsters is different from that of small lobsters. The blending of DMR and NMFS data into a single set of data may be a solution. However, whether such a set of data can describe the dynamics of the whole population depends upon whether the lobster population dynamics are consistent among different size classes and whether there is a large difference in gear selectivity and in catchability of the trawl gear. Large differences in the population dynamics of large and small lobsters and in the gear selectivity and catchability of DMR and NMFS trawls may make such blended data less desirable for describing the population dynamics with DMR-NMFS blended data. For example, a significant increase or decrease in the abundance of lobsters of prerecruiting sizes may not be well defined with the NMFS surveys or DMR-NMFS blended data. We recommend both sets of data from the DMR inshore and the NMFS surveys be used separately in the stock assessment to define the population dynamics of the lobsters in the Gulf of Maine more accurately.

Many fish species in the world exhibit a size-dependent distribution pattern similar to that of the American lobster in the Gulf of Maine. Their population abundance and size structure are often assessed by fisheries-independent survey programs. The information is then used in the assessment and management of these fish species. Because it is often difficult with large trawls to survey inshore areas where the majority of stocks are present, the fishery-independent survey may not provide adequate coverage of inshore areas that are productive and critical, in particular, to recruitment. Thus, it is highly likely that fish in inshore areas are not adequately represented in an offshore-focused survey, and this misrepresentation would lead to errors in data on stock-size structure. Such an error may, in turn, result in large errors in a stock assessment if an age- or length-based stock assessment model is used. We suggest that two sampling programs with different spatial focuses may help identify the problems associated with a sampling program that does encompass fish

size-dependent inshore-offshore distribution (or a size-dependent distribution that is related to depth or other environmental variables). The two sampling programs can greatly improve the quality and quantity of data collected in a fishery-independent program, leading to the improved assessment and management of fisheries. In the case where only one survey program can be conducted (e.g., due to budget limitation or time constraints, or both), we suggest that area-specific fishery landing data, together with depth or other environmental variables that influence fish distributions, be used in allocating sampling efforts in a random stratified survey.

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Juvenile fish assemblages collected on unconsolidated sediments of the southeast United States continental shelf

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Abstract—Patterns were investigated in juvenile fish use of unconsolidated sediments on the southeast United States continental shelf off Georgia. Juvenile fish and environmental data were sampled at ten stations along a 110-km cross-shelf transect, including four stations surrounding Gray's Reef National Marine Sanctuary (Gray's Reef NMFS). Cross-shelf stations were sampled approximately quarterly from spring 2000 to winter 2002. Additional stations were sampled on three transects inshore of Gray's Reef NMS and four transects offshore of the Sanctuary during three cruises to investigate along-shelf patterns in the juvenile fish assemblages. Samples were collected in beam trawls, and 121 juvenile taxa, of which 33 were reef-associated species, were identified. Correspondence analysis on untransformed juvenile fish abundance indicated a cross-shelf gradient in assemblages, and the station groupings and assemblages varied seasonally. During the spring, fall, and winter, three cross-shelf regions were identified: inner-shelf, mid-shelf, and outer-shelf regions. In the summer, the shelf consisted of a single juvenile fish assemblage. Water depth was the primary environmental variable correlated with cross-shelf assemblages. However, salinity, density, and water column stratification also correlated with the distribution of assemblages during the spring, fall, and winter, and along with temperature likely influenced the distribution of juvenile fish. No along-shelf spatial patterns were found in the juvenile fish assemblages, but the along-shelf dimension sampled was small (~60 km). Our results revealed that a number of commercially and recreationally important species used unconsolidated sediments on the shelf off Georgia as juvenile habitat. We conclude that management efforts would be improved through a greater recognition of the importance of these habitats to fish production and the interconnectedness of multiple habitats in the southeast U.S. continental shelf ecosystem.

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Fisheries management has been rapidly evolving for the southeast United States with the development of a formal stock assessment process (i.e., SEDAR[1]), and the identification of essential fish habitat (EFH; SAFMC, 1998). Single-species management plans have been used as the basis for fisheries management along the southeast United States for decades, and EFH is just beginning to be incorporated into management plans (e.g., *Sargassum* and *Oculina* bank habitat of particular concern: FR, 2000). Globally, as fish stocks continue to decline (Botsford et al., 1997) and harvest shifts to new species (Pauly et al., 1998), interest in ecosystem management and implementation of MPAs in many areas, including the southeast U.S. continental shelf, is growing (SAFMC, 2001). Understanding how juvenile fish use habitat is important for both single-species fishery management and ecosystem approaches to fishery management. A description of habitat needs at each life history stage for species that represent the "significant food web" are also a recognized part of fishery ecosystem plans (NOAA[2]; CFEPTAP, 2004).

Adult population variability, in most species, is caused by variability in the survival of the early-life stages: egg, larval, and juvenile (Sissenwine, 1984; Rothschild, 1986). Survival of these early stages is influenced by both habitat quantity and quality (Gibson, 1994; Peterson, 2003). One approach to improving marine fisheries management is to incorporate habitat effects on early-life stage survival into stock assessments (Beck et al., 2001; Peterson, 2003). Determining how juvenile fish use habitat also benefits marine protected area (MPA) design and implementation. MPAs have been proposed to complement traditional fishery management practices by imposing site-specific rules to protect enclosed resources (Parrish, 1999; Dayton et al., 2000; Beck and Odaya, 2001). In some instances, MPAs provide benefits to outside areas through spillover effects (Roberts et al., 2001). MPAs may also provide a source of recruits to other nonprotected areas through planktonic transport (Cowen et al., 2000; Shanks et al., 2003). To be effective in protecting fishery resources, an MPA or a network of MPAs needs to encompass the habitats used by spe-

[1] SEDAR (Southeast Data Assessment and Review). 2002. Southeast Data Assessment and Review (SEDAR). South Atlantic Fishery Management Council, Charleston, SC. Website: http://www.sefsc.noaa.gov/sedar.jsp [Accessed on 19 October 2004.]

[2] NOAA (National Oceanic and Atmospheric Administration). 1999. Ecosystem-based fishery management. A report to Congress by the Ecosystems Principles Advisory Panel. [Available from National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA.]

cies throughout their life cycle (Browman and Konstantinos, 2004).

Habitat use by juvenile fish has been studied extensively along the southeast United States, particularly for commercial and recreational species and structured habitats. For example, there is information available regarding the habitat use of specific estuarine (e.g., *Cynoscion nebulosus*: Thayer et al., 1999; paralichthid flounders: Walsh et al., 1999) and reef-associated species (e.g., *Mycteroperca microlepis*: Ross and Moser, 1995; *Lutjanus griseus*: Chester and Thayer, 1990; *Centropristis striata*: Lehnert and Allen, 2002). The importance of structural habitat to juvenile fish is also demonstrated by the consistent use of seagrass (Thayer et al., 1999), oyster reef (Meyer and Townsend, 2000), pelagic *Sargassum* (Moser et al., 1998), mangrove (Thayer et al., 1987), marsh (Hettler, 1989), and rocky reefs (Lindeman et al., 2000). Unconsolidated sediments in estuarine systems and from the surf-zone out into shallow coastal areas (<10 m) are also important juvenile habitat (estuarine: Burke et al., 1991; Walsh et al., 1999; surf-zone: Wenner and Sedberry, 1989; Ross and Lancaster, 2002).

Despite this large body of research, little is know about the use of unconsolidated sediments on the inner-, mid-, and outer-shelves of the southeast U.S. continental shelf. Unconsolidated sediment is not identified as EFH of the southeast U.S. (SAFMC, 1998), although it is identified in several fishery management plans (red drum, snapper-grouper, rock shrimp, royal red shrimp, coastal migratory pelagics, golden crab, spiny lobsters, and calico scallops). The only offshore habitats that currently have specific protection through their own fishery management plans are coral, coral reefs, live hard bottom, and pelagic *Sargassum* (Barnette[3]; FR, 2000). Research in other shelf ecosystems has identified unconsolidated sediments on the shelf as important juvenile habitat (Norcross et al., 1997; Steves et al., 2000; Sullivan et al., 2000; Johnson et al., 2001). Further, unconsolidated sediments on the southeast U.S. continental shelf cover a majority of the area (~60–80%; Parker et al., 1983), and likely serve as juvenile habitat for the more than 1200 fish species reported from the ecosystem (estimated from Kendall and Matarese, 1994). Thus, well-defined use of unconsolidated sediment habitat by juvenile fish is needed for sediment habitat to be incorporated into future fishery resource management on the southeast U.S. continental shelf.

Our purpose was to document juvenile fish habitat use and to examine the structure of juvenile fish assemblages in unconsolidated sediments off the Georgia coast region of the southeast U.S. continental shelf. Specific objectives were 1) to provide a list of species that use unconsolidated sediments on the shelf as juvenile habitat; 2) to determine which reef-associated species use unconsolidated sediments as juvenile habitat; and 3) to describe the relationships between the juvenile assemblages and environmental factors. The focus on reef fish was motivated largely by the emphasis to protect reef areas by using MPAs, as part of the management of the reef-associated snapper-grouper complex (SAFMC, 2001). If species of the reef-associated snapper-grouper complex use unconsolidated sediments during the juvenile stage, then protection of these habitats should be incorporated into the larger MPA effort.

Materials and methods

Study site

The continental shelf off the Georgia coast is the widest part (200 km) of the southeast shelf. The shelf in this region is gently sloping and comprises four depth zones (Menzel, 1993); inner-shelf (0–20 m); mid-shelf (20–40 m), outer-shelf (40–70 m), and shelf-edge (70–200 m). Each zone has different physical dynamics (Atkinson et al., 1985), climate (Blanton et al., 2003), and larval fish assemblages (Marancik et al., 2005). Demersal habitat consists of unconsolidated sediments, primarily medium to coarse quartz and carbonate sands (Nelson et al., 1999) interspersed with rocky reefs (Parker et al., 1983). Reefs of the southeast U.S. shelf range from no relief patchy live-bottom communities, to high-relief ledges. On the shelf off Georgia, rocky-reefs cover about 30% of the bottom (Parker et al., 1983). Rocky-reef habitats have a high diversity of invertebrate (Wenner et al., 1983) and vertebrate faunas (Chester et al., 1984; Parker and Mays, 1998; Lindeman et al., 2000). Located within the continental shelf off Georgia is the 56-km^2 Gray's Reef National Marine Sanctuary (Gray's Reef NMS, Fig. 1). The sanctuary depth ranges from 18 to 22 m and the benthic macrohabitat is mainly (~75%) unconsolidated sand sediments interspersed with patchy live-bottom and moderate relief (≤2 m) hard-bottom ledges (Parker et al., 1994). The adult reef fish community of the sanctuary is typical of other inner-shelf (<30 m) reefs of the South and North Carolina shelves (Chester et al., 1984; Parker et al., 1994).

Collection of juvenile fish and environmental data

Sampling of juvenile fish was conducted approximately quarterly from April 2000 through February 2002 (Table 1). Ten cross-shelf stations (stations 1–7), approximately 18.5 km apart, were sampled during most cruises (Fig. 1). Stations were missed on some cruises owing to weather and equipment failure (Table 1). The cross-shelf transect was 110 km long and stations were sampled on the inner-, mid-, and outer-shelf (10 to 50 m water depth). To avoid sampling within Gray's Reef NMS, four stations were placed immediately adjacent to the four sides of the sanctuary (stations 2.1–2.4). Additional

[3] Barnette, M. C. 2001. A review of the fishing gear utilized within the Southeast Region and their potential impacts on essential fish habitat. NOAA Tech. Memo. NMFS-SEFSC-449, 62 p. [Available from National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA.]

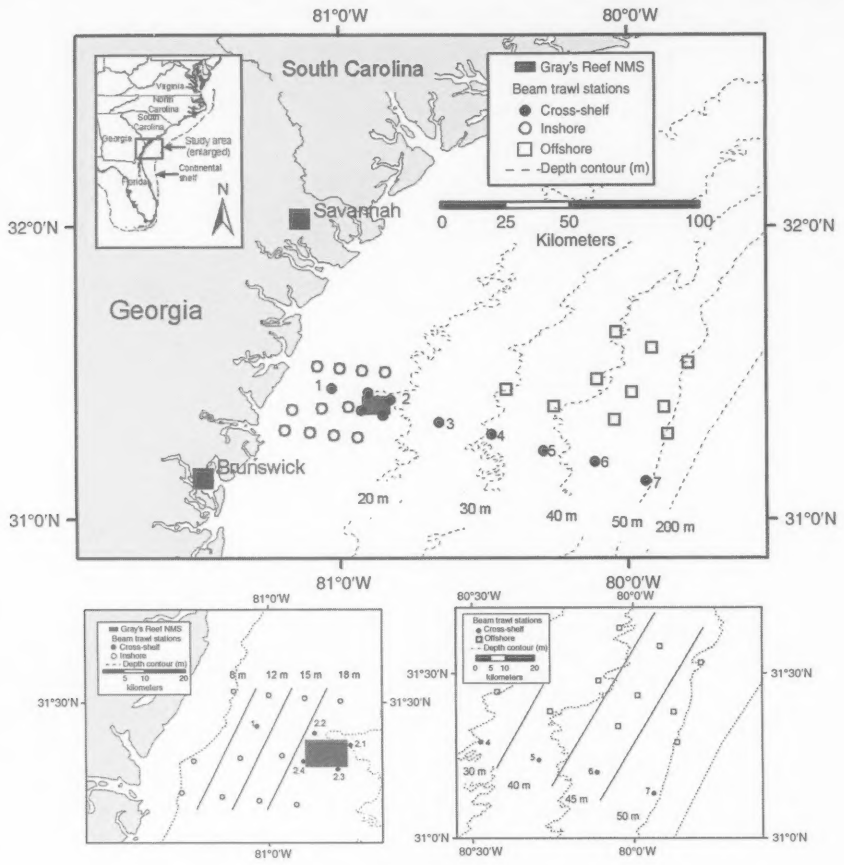

Figure 1

Maps of the study area showing cross-shelf beam trawl stations (black dots) at which juvenile fish and environmental sampling occurred quarterly from April 2000–February 2002 (see Table 1). Additional stations were sampled inshore (circles) and offshore (squares) of Gray's Reef NMS (see Table 1) (**A**). Station groups used in correspondence analysis of the inshore (**B**) and offshore (**C**) juvenile fish data sets are separated by solid lines and labeled.

stations were sampled along three transects inshore of Gray's Reef NMS and along four offshore transects during three cruises (Fig. 1 and Table 1). The benthic macrohabitat of the cross-shelf stations was determined by using a remotely operated vehicle (ROV, *Phantom* S2). The ROV was deployed off the starboard side of the ship. Approximately 30 m of the ROV tether was let out before the tether was attached to a 45-kg weight. The weight was lowered to 3 m above the bottom and the ship was allowed to drift. During April 2000, two 15-min drifts were made at eight of the ten cross-shelf stations. Because of inclement weather, no drifts were conducted at the inner- or outer-most stations. Video (HI-8 mm) was recorded of each drift.

Concurrent fish and hydrographic measurements were taken to examine the relationship between juvenile fish assemblages and the environment. At each station, temperature, salinity, density, and water depth were measured from the water's surface to one meter above the bottom with a Seabird CTD (SBE19, Seabird Electronics, Inc., Bellevue, WA). Juvenile fish were collected at each station by using a 2-m beam trawl with a 6-mm mesh body and a 3-mm mesh tail bag. Two kilogram weights were added to each skid of a standard 2-m beam trawl (Kuipers, 1975) to ensure that the trawl stayed on the bottom. Three 5-min bottom tows were made at each station. Samples were sorted on deck and fish and invertebrates were preserved in 95% ethanol.

Preparation of fish data

All beam trawl samples were sorted and fish were identified to the lowest possible taxonomic level by using previously published descriptions (e.g., Able and Fahay,

Table 1

Number of beam trawl stations sampled off the coast of Georgia for juvenile fish. Station groups correspond to the station locations in Figure 1.

				Station group		
Year	Month	Dates	Season	Cross-shelf	Inshore	Offshore
2000	April	24–27	spring	9		
	June	19–22	summer	9		
	August	15–17	summer	9		
	October	03–07	fall	10		
2001	Jan/Feb	30–01	wnter	10		
	March	21–23	winter	10		
	Apr/May	30–04	spring	10	11	
	June	04–09	summer	10		
	August	03–06	summer	10		
	September	07–09	fall			6
	October	11–13	fall	10		
2002	February	08–13	winter	10	10	10

1998; Munroe, 1998). Identification to species was not possible for all fish. Taxa were separated into larval, juvenile, and adult age classes according to life-history characteristics from the literature and standard length distributions (SL, mm) from the beam trawl collections. Fish were classified as larvae if fin development was not complete (and defined as "larvae at settlement stage" when fin formation was complete and pigmentation incomplete; fish were classified as juveniles when pigmentation was complete, and were classified as adults if they exceeded the reported minimum SL for sexual maturity. Because the mesh of the beam trawl is large enough to extrude larvae and less efficient at catching large (>150 mm SL) fish (Kuipers, 1975), analyses in this study were conducted only on settlement and juvenile stages, which were combined and referred to as juveniles. Taxa were also classified as not reef-associated, strongly reef-associated or weakly reef-associated based on habitat-use information (Chester et al., 1984; Humann, 1994; Parker et al., 1994; Parker and Mays, 1998; Lindeman et al., 2000). Taxa found predominantly on rocky-reefs (e.g., *Centropristis striata*) were classified as strongly reef-associated. Those that use rocky-reefs and other habitats (e.g., *Diplectrum formosum*) were classified as weakly reef-associated.

Standard catch per unit of effort (CPUE, fish/5-min bottom tow) was calculated for each taxon. CPUE for each taxon at each station was the average of all replicate tows. Three juvenile stage data sets (i.e., cross-shelf, inshore, and offshore) were compiled by using data from the station groups (Table 1, Fig. 1): the cross-shelf data set consisted of data from stations one through seven (Fig. 1A); the inshore data set consisted of data from stations predominately inside the 20-m isobath (Fig. 1B), and the offshore data set consisted of date from stations >30 m in depth (Fig. 1C).

For statistical analyses, the three data sets were subdivided into two groups so that rare taxa would not greatly influence the classification of assemblages. The first group consisted only of abundant taxa, and the second group included abundant and rare taxa. Cross-shelf and offshore data sets were subdivided into taxa that made up at least 10% (abundant) and 1% (abundant and rare) of the collections at any one station. The inshore data set was subdivided at 5% and 1% levels. The data sets were further condensed by eliminating all taxa not identified to species level with the exception of *Stenotomus* sp. and *Rypticus* sp. which were probably single species. Several abundant taxa were excluded from the analysis because they were only identified to genus and have multiple species common in the area, and each species may differ in distribution: i.e., *Etropus* spp. (4 species), *Prionotus* spp. (14 species), *Sphoeroides* spp. (11 species), *Microgobius* spp. (3 species), and *Bothus* spp. (3 species). As a result, the numbers of taxa in the rare and abundant data sets used in analyses were as follows: cross-shelf 82 (rare) and 28 (abundant) (Table 2); inshore 28 and 13 (Table 3), and offshore 51 and 17 (Table 4).

Preparation of environmental data

Season and five environmental (or hydrographic) variables were chosen in an attempt to explain variation in the juvenile fish data. Cruises were assigned to one of four seasons (Table 1) based on wind (Atkinson et al., 1985) and temperature regimes (Marancik et al., 2005). Environmental variables were calculated as in Marancik et al. (2005). Briefly, CTD measurements of temperature (°C) and salinity were used to derive density (σ_t, Kg/m^3). Bottom temperature, salinity, and density were used in the multivariate analyses with juvenile fish because benthic habitats were sampled and bottom values were

Table 2

Taxa collected by beam trawl during two years of sampling (April 2000–February 2002) at the cross-shelf stations (see Fig. 1A) off the coast of Georgia, and used in correspondence analysis. Mean and standard deviation (SD) of catch per unit of effort (CPUE) were calculated for the entire data set. Taxa that constituted one or ten percent of any one station were used in the analyses, and an asterisk next to a taxon indicates that it was in the 10% data set. Seasonal juvenile assemblages from correspondence analysis are shown (I= inner-shelf; I/M=inner-/mid-shelf; M=mid-shelf; O-outer-shelf; and S=entire shelf: S). Taxa in bold indicate reef-associated species.

		CPUE		Seasonal juvenile assemblage			
Family	Taxon	Mean	SD	Spring	Summer	Fall	Winter
Elopidae	*Elops saurus*	0.010	0.097		S		M
Ophichthidae	*Ophichthus gomesii*	0.003	0.056		S		
	Ophichthus ocellatus	0.021	0.155	I/M	S	M	M
Congridae	*Ariosoma balearicum**	0.163	0.479	I/M	S	M	M
Clupeidae	*Brevoortia tyrannus*	0.019	0.134				M
	Etrumeus teres	0.006	0.080				M
	Sardinella aurita	0.003	0.056		S		
Engraulididae	*Anchoa hepsetus**	0.303	1.313	I/M	S	M	
	Anchoa lamprotaena	0.013	0.237			M	
Argentinidae	*Argentina striata*	0.006	0.080				O
Synodontidae	*Synodus foetens**	0.746	1.626	I/M, O	S	M	M
	Synodus poeyi	0.048	0.487	O			
	Trachinocephalus myops	0.103	0.388	O	S	M,O	O
Gadidae	*Urophycis regia**	0.234	0.759	I/M			M
Ophidiidae	*Ophidion selenops**	2.185	4.844	I/M	S	M	M
	*Otophidium omostigmum**	0.364	1.190	O	S	O	M
Batrachoididae	*Porichtys plectrodon*	0.006	0.072			M	
Antennariidae	***Antennarius radiosus***	0.006	0.080				M, O
Ogcocephalidae	*Halieutichthys aculeatus*	0.019	0.137	O	S		
	Ogcocephalus nasutus	0.024	0.150	I/M	S		I
Exocoetidae	*Hemiramphus brasilieusis*	0.003	0.056		S		
	Hirundichthys affinis	0.022	0.169		S	M	
Syngnathidae	*Hippocampus erectus*	0.052	0.233	I/M	S	M	I,M,O
	*Syngnathus scovelli**	0.025	0.192		S	I	
	Syngnathus springeri	0.025	0.158	I/M	S		M
Scorpaenidae	***Scorpaena dispar***	0.019	0.161		S	M	
	Scorpaena plumieri*	0.080	0.332	I/M	S	M	M
Triglidae	*Bellator brachychir*	0.006	0.074		S	O	
	Bellator militaris	0.006	0.073	O	S		
	*Prionotus carolinus**	1.047	2.970	I/M	S	M	M
	Prionotus ophryas	0.003	0.056		S		
	Prionotus scitulus	0.022	0.146				M
Serranidae	***Centropristis ocyurus****	0.167	1.344	O	S		O
	Centropristis striata	0.022	0.186	I/M	S		
	Diplectrum formosum*	1.556	3.659	O	S	M,O	M
	Serraniculus pumilio	0.180	0.817	I/M	S	M	M
	Serranus phoebe*	0.085	0.466	O	S	O	O
Priacanthidae	***Priacanthus arenatus***	0.003	0.056		S		
Apogonidae	***Pristigenys alta***	0.016	0.123		S	M	
	Apogon pseudomaculatus	0.012	0.108		S		
Carangidae	*Caranx bartholomaei*	0.003	0.051	O			
	Chloroscombrus chrysurus	0.014	0.124		S	M	
	Decapterus macarellus	0.006	0.080	O			M
	*Decapterus punctatus**	0.022	0.218		S		M
	Trachurus lathami	0.003	0.054	I/M			
Lutjanidae	***Lutjanus analis***	0.003	0.056		S		

continued

Table 2 (continued)

Family	Taxon	CPUE		Seasonal juvenile assemblage			
		Mean	SD	Spring	Summer	Fall	Winter
Haemulidae	***Haemulon aurolineatum****	0.022	0.214		S	O	
Sparidae	***Stenotomus* sp.***	0.119	0.811	I/M	S	M	M
	Lagodon rhomboides	0.016	0.149				M
Sciaenidae	*Cynosion nothus*	0.009	0.110			M	
	Cynosion regalis	0.011	0.118			M	
	*Larimus fasciatus**	0.036	0.363			I	
	*Leiostomus xanthurus**	0.621	3.752				I
	Menticirrhus americanus	0.013	0.185			I	
Mugilidae	*Mugil cephalus**	0.051	0.469				O
	Mugil curema	0.010	0.099				M
Labridae	***Halichoeres bivattatus***	0.006	0.113		S		
	Xyrichtys novacula	0.003	0.056		S		
Uranoscopidae	*Kathetostoma albigutta*	0.003	0.056				M
Dactyloscopidae	*Dactyloscopus moorei**	0.837	2.329	I/M	S	M	M
Blenniidae	*Hypleurochilus geminatus*	0.010	0.126	I/M	S		
	Parablennius marmoreus*	0.006	0.080		S		
Callionymidae	*Diplogrammus pauciradiatus**	0.025	0.174		S		
Gobiidae	*Ioglossus calliurus*	0.003	0.047	O	S		
Stromateidae	*Peprilus triacanthus*	0.038	0.218	I/M			M
Bothidae	*Bothus lunatus*	0.006	0.080	O			O
	Bothus ocellatus	0.021	0.155		S	M	O
	*Bothus robinsi**	0.497	1.643	O	S	M	O
Paralichthyidae	*Ancylcopsetta quadrocellata*	0.063	0.300				M
	Citharichthys macrops	0.096	0.475	I/M	S	M	M
	Cyclopsetta fimbriata	0.031	0.184	O	S	M	
	*Syacium papillosum**	0.042	0.282	I/M	S	O	
Soleidae	*Gymnachirus melas**	0.019	0.134	O	S		
Cynoglossidae	*Symphurus diomedeanus*	0.010	0.126	O			
	*Symphurus minor**	0.519	1.801	O	S	O	O
	Symphurus parvus	0.016	0.125	O	S		
	Symphurus plagiusa	0.006	0.113		S		
	*Symphurus urospilus**	0.324	0.806	I/M	S	M	M
Balistidae	***Aluterus schoepfi***	0.006	0.076		S	M	
	*Monocanthus hispidus**	0.504	1.424	I/M	S	M	O
	Monocanthus setifer	0.003	0.047			O	
Ostraciidae	***Lactophyrs quadricornis***	0.005	0.068		S		

highly correlated with the average water column and surface variables (Marancik et al., 2005). Vertical stratification was estimated by using Simpson's stratification parameter (Simpson and James, 1985), Φ (joules/m^3), which is a measure of the resistance of water to mixing (higher numbers signify higher resistance to mixing).

Data Analyses

Correspondence analysis (CA) and canonical correspondence analysis (CCA) were performed by using the statistical package CANOCO (ter Braak and Smilauer, 2002). Using the cross-shelf, inshore, and offshore data sets, we had seven objectives: 1) to describe the cross-shelf patterns in the juvenile fish assemblages; 2) to identify the species associated with the assemblages including the reef-associated species that contribute to these assemblages; 3) to describe the relationship among cross-shelf patterns in the assemblages and environmental variables; 4) to describe the seasonal patterns in the assemblages; 5) to determine the relation between seasonal assemblages and environmental variables; 6) to describe the along-shelf and cross-shelf patterns in the inshore data set; and 7) to describe the along-shelf and cross-shelf patterns in the offshore data set.

Correspondence analysis was used to define juvenile fish assemblages and to explore the factors that influence distribution of assemblages on the shelf off

Table 3

Taxa collected by beam trawl during spring and winter sampling at stations inshore of Gray's Reef National Marine Sanctuary (see Fig. 1B), and used in correspondence analysis. Mean and standard deviation (SD) of catch per unit of effort (CPUE) were calculated for the entire data set. Taxa that constituted one or five percent of any one station were used in the analyses, and an asterisk next to a taxon indicates that it was in the 5% data set. Station depth groups for each season for juvenile assemblages, as determined from correspondence analysis, are shown. Taxa in bold indicate reef-associated species.

		CPUE		Juvenile assemblage	
Family	Taxon	Mean	SD	Spring	Winter
Congridae	*Ariosoma balearicum**	0.022	0.147	12–15 m	
Clupeidae	*Sardinella aurita*	0.011	0.105	12–15 m	
Engraulididae	*Anchoa hepsetus**	0.613	5.135	8 m	8 m
	Anchoa lamprotaena	0.022	0.147	8 m	8 m
Synodontidae	*Synodus foetens**	0.416	1.525	12–15 m	12–15 m
Gadidae	*Urophycis regia**	0.718	1.176	12–15 m	12–15 m
Ophidiidae	*Ophidion selenops**	0.794	1.867	12–15 m	12–15 m
Ogcocephalidae	*Ogcocephalus nasutus*	0.011	0.105	12–15 m	
Syngnathidae	*Hippocampus erectus*	0.098	0.333	12–15 m	12–15 m
Scorpaenidae	***Scorpaena plumieri***	0.044	0.206	12–15 m	12–15 m
Triglidae	*Prionotus carolinus**	2.227	2.939	12–15 m	12–15 m
	Prionotus scitulus	0.122	0.332	12–15 m	
Serranidae	***Centropristis ocyurus***	0.022	0.147	12–15 m	
	Diplectrum formosum*	0.099	0.366	12–15 m	12–15 m
Sparidae	***Stenotomus* sp.***	0.261	0.949	12–15 m	12–15 m
Sciaenidae	*Leiostomus xanthurus**	14.053	46.103	8 m	
	Stellifer laceolatus	0.022	0.210	8 m	
Mullidae	*Upeneus parvus*	0.011	0.105	12–15 m	
Dactyloscopidae	*Dactyloscopus moorei**	0.545	1.108	12–15 m	12–15 m
Gobiidae	*Gobiosoma bosci*	0.053	0.303	12–15 m	
Stromateidae	*Peprilus triacanthus**	0.076	0.370	12–15 m	
Bothidae	*Bothus ocellatus*	0.010	0.095	12–15 m	
Paralichthyidae	*Ancylcopsetta quadrocellata*	0.344	0.604	8 m	12–15 m
	Citharichthys macrops	0.088	0.353	8 m	12–15 m
Cynoglossidae	*Symphurus plagiusa**	0.112	0.538	8 m	
	Symphurus urospilus	0.109	0.403	12–15 m	12–15 m
Balistidae	*Monocanthus hispidus**	0.154	0.445	12–15 m	12–15 m
Ostraciidae	***Lactophyrs quadricornis***	0.011	0.105	8 m	

the coast of Georgia. CA arranges stations and species along gradients (Greenacre, 1984), creating a low-dimensional map (an ordination). Points that occur in close proximity can be considered to have similar species composition and abundance. Points that occur on the same dimension define gradients in the data. The eigenvalues, which are a relative measure of the amount of variance explained by each CA dimension (ter Braak and Smilauer, 2002), were used to determine the number of dimensions that best described the data. CA on untransformed CPUE data was used to define assemblages for the cross-shelf, inshore, and offshore data sets in relation to season.

Canonical correspondence analysis, which incorporates environmental variables by aligning species and station data along environmental gradients, was used to explore the relationship between juvenile assemblages and the environment. The species-environment correlation is a measure of the strength of the relation between the species data and the environmental data for each CCA dimension (ter Braak and Smilauer, 2002). The product of the species-environment correlation and the eigenvalue can be used to describe the variance in the data. CCA on untransformed CPUE data and standardized environmental data was used to explore the relation between the assemblages and environment for the cross-shelf, inshore, and offshore data sets in relation to season. Environmental data were standardized to a mean of zero and a standard deviation of one.

Results

Habitat characterization

The environmental variables showed the typical seasonal and depth patterns across the shelf (Fig. 2). During the spring, bottom water temperature was near 20°C

Table 4

Taxa collected by beam trawl during fall and winter sampling at stations offshore of Gray's Reef National Marine Sanctuary (see Fig. 1C), and used in correspondence analysis. Mean and standard deviation (SD) of catch per unit of effort (CPUE) were calculated for the entire data set. Taxa that constituted one or ten percent of any one station were used in the analyses, and an asterisk next to a taxon indicates that it was in the 10% data set. Station depth groups for each season for juvenile assemblages, as determined from correspondence analysis, are shown. Taxa in bold indicate reef-associated species.

		CPUE		Juvenile assemblage	
Family	Taxon	Mean	SD	Spring	Winter
Muraenidae	*Gymnothorax saxicola*	0.024	0.155	50 m	
Ophichthidae	*Ophichthus gomesii*	0.036	0.246	30–45 m	
Congridae	Ariosoma balearicum	0.183	0.485	30–45 m	50 m
Synodontidae	*Synodus foetens**	0.410	0.980	30–45 m	30–45 m
	Trachinocephalus myops	0.147	0.391	30–45 m	30–45 m
Ophidiidae	*Ophidion selenops**	0.813	2.289	30–45 m	30–45 m
	*Otophidium omostigmum**	0.957	1.401	50 m	30–45 m
Batrachoididae	*Porichtys plectrodon*	0.012	0.105	50 m	
Antennariidae	***Antennarius ocellatus***	0.012	0.109	50 m	
	Antennarius radiosus	0.039	0.203	50 m	
Ogcocephalidae	*Ogcocephalus nasutus*	0.059	0.233	30–45 m	
	Ogcocephalus parvus	0.024	0.154	50 m	
Syngnathidae	*Hippocampus erectus*	0.024	0.154	50 m	50 m
Scorpaenidae	***Scorpaena dispar****	0.152	0.989	50 m	50 m
	Scorpaena plumieri	0.137	0.510	30–45 m	50 m
Triglidae	*Bellator brachychir*	0.012	0.109	30–45 m	
	*Prionotus carolinus**	0.360	0.785	30–45 m	30–45 m
	Prionotus scitulus	0.024	0.153	30–45 m	
Serranidae	***Centropristis ocyurus****	0.998	2.381	50 m	50 m
	Diplectrum formosum*	2.305	4.132	30–45 m	30–45 m
	Epinephelus niveatus	0.024	0.155	50 m	30–45 m
	Serraniculus pumilio*	0.206	0.579	50 m	30–45 m
	Serranus phoebe*	0.857	3.515	50 m	50 m
	Serranus subligarius	0.037	0.331	50 m	
Grammistidae	***Rypticus* sp.***	0.239	1.330	50 m	50 m
	Rypticus bistrispinus*	0.280	1.336	50 m	
Priacanthidae	***Pristigenys alta***	0.049	0.217	50 m	
Apogonidae	***Apogon maculatus****	0.314	1.525	50 m	
	Apogon pseudomaculatus	0.107	0.439	30–45 m	50 m
Haemulidae	***Haemulon aurolineatum***	0.035	0.233	30–45 m	
Sparidae	***Stenotomus* sp.**	0.049	0.267	30–45 m	
Mugilidae	*Mugil cephalus*	0.024	0.221	30–45 m	
	Mugil curema	0.037	0.192	30–45 m	
Labridae	***Halichoeres bivittatus***	0.015	0.133	50 m	
Dactyloscopidae	*Dactyloscopus moorei*	0.049	0.266	30–45 m	
Blenniidae	*Hypleurochilus geminatus*	0.073	0.262	50 m	50 m
	Parablennius marmoreus	0.024	0.221	30–45 m	
Callionymidae	*Diplogrammus pauciradiatus*	0.048	0.212	50 m	30–45 m
Bothidae	*Bothus ocellatus**	0.143	0.701	30–45 m	50 m
	*Bothus robinsi**	0.665	1.145	30–45 m	30–45 m
Paralichthyidae	*Ancylcopsetta quadrocellata*	0.060	0.284	30–45 m	
	Citharichthys macrops	0.024	0.152	30–45 m	
	Cyclopsetta fimbriata	0.095	0.394	30–45 m	
	Syacium papillosum	0.087	0.329	30–45 m	
Cynoglossidae	*Symphurus minor**	2.047	3.593	30–45 m	30–45 m
	*Symphurus urospilus**	0.194	0.501	30–45 m	30–45 m
Balistidae	***Cantherhines pullus***	0.061	0.287	50 m	
	Monocanthus ciliatus	0.012	0.110	50 m	
	*Monocanthus hispidus**	0.891	2.057	30–45 m	30–45 m
	Monocanthus setifer	0.012	0.110	50 m	
Ostraciidae	***Lactophyrs quadricornis***	0.012	0.108	30–45 m	

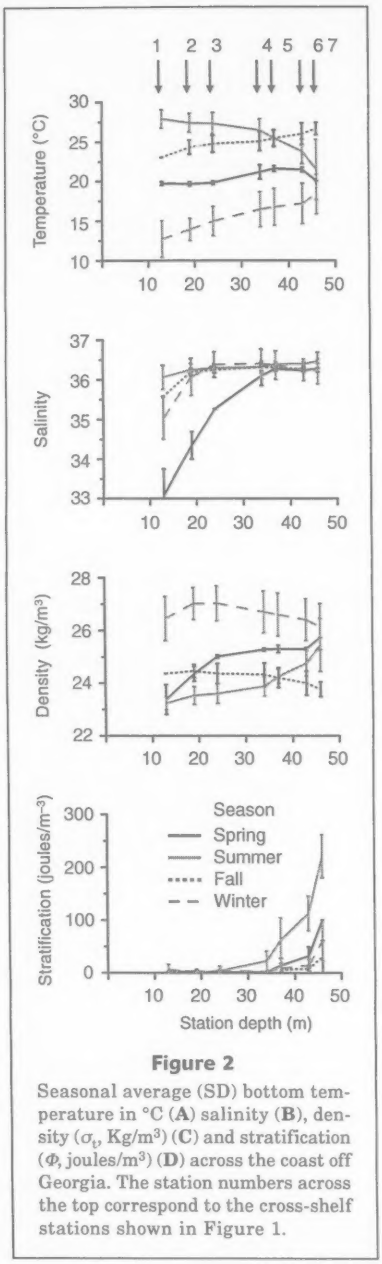

Figure 2

Seasonal average (SD) bottom temperature in °C (**A**) salinity (**B**), density (σ_t, Kg/m^3) (**C**) and stratification (Φ, joules/m^3) (**D**) across the coast off Georgia. The station numbers across the top correspond to the cross-shelf stations shown in Figure 1.

across the entire shelf (Fig. 2A). In the summer, bottom waters were warmest on the inner-shelf and cooler on the outer-shelf (Fig. 2A). Average fall bottom water temperature was intermediate between that of spring and summer and was warmest on the outer-shelf (Fig. 2A). The lowest bottom water temperatures were measured during the winter on the inner-shelf (Fig. 2A). Bottom salinity was lowest on the inner-shelf during all seasons (Fig. 2B). Bottom salinity was also low on the inner- and mid-shelves during the spring and rose to near 36 psu on the outer-shelf (Fig. 2B). During the summer, fall, and winter the mid- and outer-shelf bottom salinities remained near 36 psu (Fig. 2B). Bottom water density reflected the seasonal and cross-shelf patterns in temperature and salinity (Fig. 2C). Bottom water density was greatest during the winter when water temperatures were the lowest (Fig. 2C). In the spring, a cross-shelf pattern occurred and density was lowest inshore (Fig. 2C). During summer bottom water density was lowest inshore, where the water temperatures were low (Fig. 2C). There was no cross-shelf density pattern in the fall (Fig. 2C). Water column stratification was greatest during the summer and Simpson's stratification numbers were always higher on the outer-shelf (Fig. 2D). The low stratification numbers on the inner- and mid-shelves (Fig. 2D) indicated that there was a high probability of vertical mixing in all seasons.

Video analysis of the ROV tows indicated that the cross-shelf bottom on the continental shelf off the coast of Georgia consisted predominately of unconsolidated sand sediments. Hard-bottom (rock rubble) was seen in one video frame at station 2.3, on the southern edge of the Gray's Reef NMS. No ROV data were collected at the inner- or outermost stations; however, no hard-bottom has been reported from either station (SEAMAP-SA, 2001). These data indicate that unconsolidated sediments were sampled.

Cross-shelf patterns in the juvenile fish assemblages

One hundred eighty one taxa were collected with the beam trawl, and of these, 121 were classified as juveniles (Appendix). Twelve strongly reef-associated species and 21 weakly reef-associated species were collected. The assemblages exhibited a cross-shelf gradient and the station groupings varied seasonally (Fig. 3). CA of both the 10% and 1% data sets resulted in the same overall patterns; however, rare species frequently were found between station groups in the ordination. During the spring, there were two cross-shelf station groups that overlapped at station 4 (Fig. 3A), an inner and mid-shelf group (stations 1–4), mainly inshore of the 30-m isobath, and an outer-shelf group (stations 4–7). Eigenvalues of the CA showed that the separation of the two groups was mainly along the first dimension (Table 5). During the summer, there was little cross-shelf pattern in the assemblages. The only exception was station 2.3, which was distinct from all other stations because live bottom was sampled at that station (Fig. 3B). When this station was removed from the analysis, there was no cross-shelf pattern in the summer data set (Fig. 3C). Three cross-shelf station groups were present in the fall (Fig. 3D): inner-shelf (station 1), mid-shelf (stations 2–5), and outer-shelf (stations 6–7). Separation of the groups was predominately along the first dimension (Table 5), which separated the inner- and mid-shelf station groups from the outer-shelf station group. During the winter, there was a cross-shelf gradient in the juvenile fish assem-

blages and the three overlapping groups were not as distinct as they were in spring or fall (Fig. 3, E and F). The species composition and abundance at station 7 from January 2001 was dissimilar from the rest of the winter samples (Fig. 3E) and generated a high eigenvalue for the second dimension (Table 5). The cross-shelf gradient along the first dimension had the highest eigenvalue (Table 5) in the winter; the first dimension showed three cross-shelf groups (Fig. 3F): an inner-shelf group (stations 1–2), a mid-shelf group (stations 2–5), and an outer-shelf group (stations 4–7).

Species composition of juvenile fish assemblages

Five seasonal juvenile fish assemblages were associated with the cross-shelf station groups (Fig. 4, Table 2). The cross-shelf distribution of juveniles was related to adult distribution patterns and habitat use (Appendix). During the spring, the inner- and mid-shelf assemblage comprised species that are found in estuarine, coastal, and open-shelf habitats as adults (Fig. 4A, Appendix). *Synodus foetens* fell between the two station groups on the ordination, and was found in the both assemblages (Fig 4A). The outer-shelf assemblage was predominately coastal and open-shelf species (Fig. 4A, Appendix). Three reef-associated species were present in both assemblages (Table 2).

Eighteen juvenile reef-associated species were collected during the summer (Table 2). Six species (*Centropristis striata, Apogon pseudomaculatus, Haemulon aurolineatum, Stenotomus* sp., *Halichoeres bivittatus,* and *Parablennius marmoreus*) were collected in June 2001 at station 2.3, when sampling was conducted over live-bottom. Sixteen of the 56 species from the summer sampling were collected solely during this season, and overall, the entire shelf was considered a single assemblage (Table 2).

Only estuarine and coastal species were observed in the inner-shelf assemblage during the fall (Fig. 4B, Table 2). The greatest number of species was observed in the mid-shelf assemblage (Fig. 4B, Table 2), which consisted of a mixture of estuarine, coastal, and open-shelf species (Fig. 4B, Appendix). *Diplectrum formosum* was collected from stations 2 to 7 and was in both the mid- and outer-shelf assemblages (Fig 4B). The outer-shelf assemblage consisted of only coastal and open-shelf species (Fig. 4B, Appendix). Seven reef-associated species were present in mid- to outer-shelf assemblages during the fall (Table 2).

There was a cross-shelf gradient in the winter juvenile fish assemblages that was in-

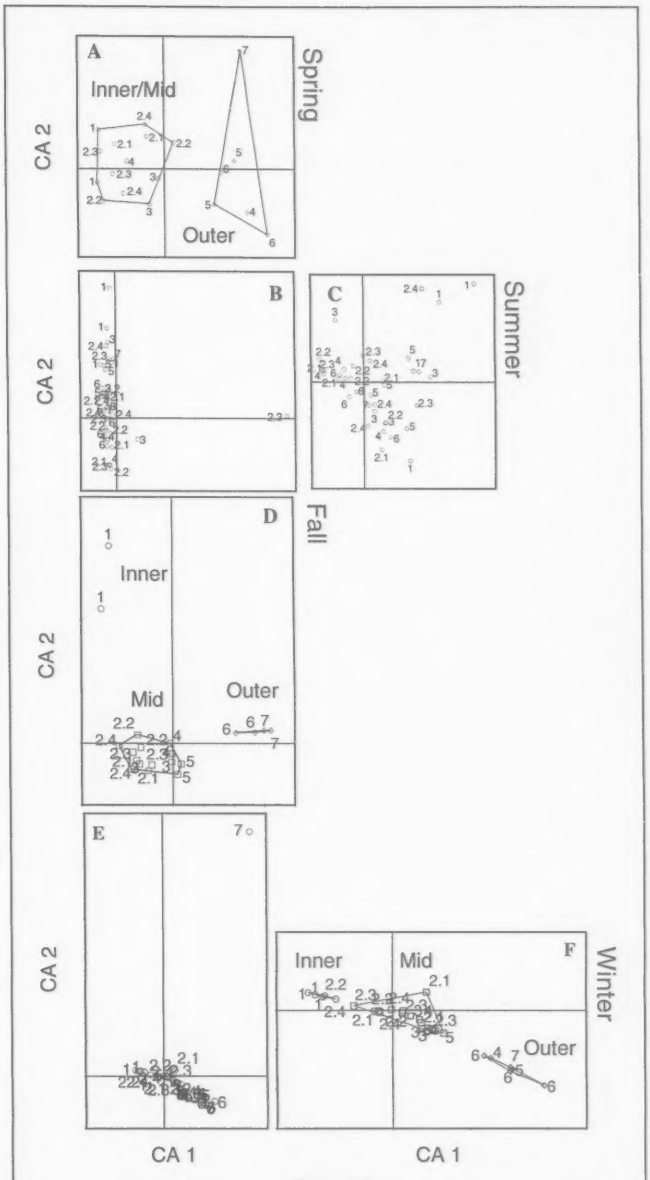

Figure 3

Correspondence analysis (CA) ordinations (portraying the first and second dimension scores) of the 10% cross-shelf data set showing station groups in each season; spring (**A**), summer (**B**), summer without station 2.3 (**C**), fall (**D**), winter (**E**), and winter without station 7 (**F**). The cross-shelf station groups were identified within each season. Solid lines enclose the boundary of each station group with three or more stations. Station groups comprising one or two stations are not enclosed by a solid line. Each station group is labeled and identified by a different symbol. The dashed lines intersect at the origin of the plot. Analyses were conducted by using juvenile fish abundance data only. Data from each cruise within a season are shown together.

Table 5

Eigenvalues and species–environment correlations ($r2$) for each axis analyzed (correspondence analysis [CA] and canonical correspondence analysis [CCA]) by season and the entire year. A sharp drop in the eigenvalue marks the axes that explain most of the data. Eigenvalues alone explain the variance in the data for CA. The product of the species-environment correlation and the eigenvalue explains the variance in the data for CCA.

		CA axis				CCA axis			
Season		1	2	3	4	1	2	3	4
Spring	Eigenvalue	0.574	0.326	0.273	0.251	0.538	0.292	0.172	0.052
	r^2					0.971	0.962	0.959	0.577
Summer	Eigenvalue	0.461	0.389	0.350	0.303	0.248	0.160	0.123	0.049
	r^2					0.857	0.742	0.684	0.422
Fall	Eigenvalue	0.604	0.376	0.275	0.224	0.447	0.301	0.121	0.090
	r^2					0.910	0.899	0.846	0.765
Winter	Eigenvalue	0.669	0.644	0.492	0.414	0.531	0.306	0.181	0.118
	r^2					0.930	0.862	0.669	0.625
Year	Eigenvalue	0.698	0.507	0.499	0.394	0.169	0.125	0.102	0.089
	r^2					0.606	0.628	0.615	0.522

Table 6

The P values from a Monte Carlo permutation test on the environmental variables for each season. Significant P values ($P<0.05$) are in bold.

	Season			
Variable	Spring	Summer	Fall	Winter
Depth (m)	**0.002**	0.918	**0.002**	**0.002**
Stratification	**0.002**	**0.002**	0.622	0.178
Bottom temperature	0.410	0.138	0.094	0.070
Bottom salinity	1.000	0.160	**0.020**	0.316
Bottom density	**0.034**	0.848	0.102	**0.020**

fluenced by the single station 7 (Fig. 3E), which had a high abundance of *Monacanthus hispidus*. *Bothus ocellatus* and *B. lunatus* were also collected only at this station (Table 2). The inner-shelf consisted of one dominant species (Fig. 4C), and the mid-shelf had the greatest number of species (Fig. 4C, Table 2). The outer-shelf assemblage consisted of estuarine, coastal, and open-shelf species (Fig. 4C). Six reef-associated species were found in the mid- and outer-shelf assemblages. The distributions of species in all three winter assemblages indicated cross-shelf mixing. An open-shelf species, *Ogcocephalus nasutus*, was present in the inner-shelf assemblage and *Mugil cephalus*, which uses estuaries as adults, was present in the outer-shelf assemblage (Table 2, Appendix). Finally, two slope species (*Argentina striata* and *Antennarius radiosus*) were present in the mid- and outer-shelf assemblages (Table 2, Appendix).

Cross-shelf patterns in juvenile fish assemblages and environmental variables

The juvenile fish assemblages and environmental variables all showed a cross-shelf gradient, which was correlated with depth during three of the four seasons (Table 6). The relationship between the other environmental variables and assemblages varied with season. In spring, the gradient in the inner- and mid-shelf and outer-shelf was mainly along the first dimension (Table 5) and was influenced by depth, bottom salinity, and bottom density (Fig. 5A). Inner- and mid-shelf stations were shallower and had less saline and dense bottom water than outer-shelf stations (Fig. 2). The spread along the second dimension in the outer-shelf group was less important (Table 5) and was influenced by the higher stratification of the outer stations (Fig. 2). A Monte Carlo permutation test of the environmental variables for spring indicated that depth, stratification, and bottom density were significantly related to the juvenile assemblages and the grouping of stations in the CCA (Table 6).

The summer juvenile assemblage was not related to these environmental variables. The ordination of the station and species data changed with the inclusion of environmental data to the analyses, indicating a poor relationship between the juvenile data and the environment (Fig. 5B) The low eigenvalues also indicated that very little of the observed variation in the data was explained by the environment (Table 5). These results agree with the results from the CA, both of which indicate that the entire shelf is a single assemblage.

The fall cross-shelf patterns in the juvenile fish assemblages were related to depth and bottom salinity (Fig. 5C). The first dimension had the highest eigenvalue and species-environment correlation (Table 5)

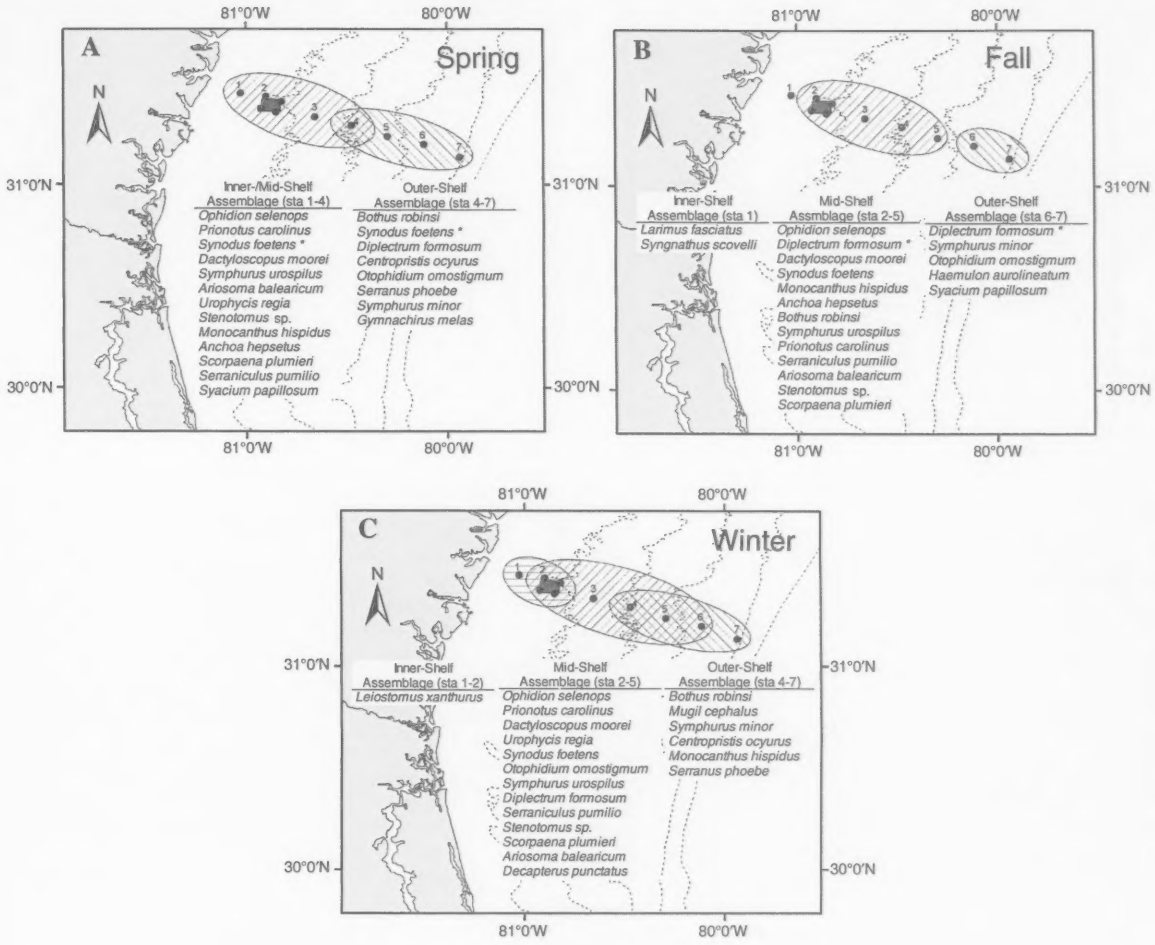

Figure 4

Cross-shelf juvenile fish assemblages identified from correspondence analysis for spring (**A**), fall (**B**), and winter (**C**). Summer was not plotted because there was only one summer assemblage. Hatched circles enclose stations that grouped together each season. Taxa from the 10% cross-shelf data set, in order of abundance, for each assemblage are shown in the tables. Asterisks indicate species that were found in multiple assemblages.

and was mainly influenced by depth; the shallower inner- and mid-shelf stations were separate from the outer-shelf stations. The second dimension was related to bottom salinity (Fig. 5C) and correlated with the separation of the inner-shelf station from the higher saline mid- and outer-shelf stations (Fig. 2). A Monte Carlo permutation test of the relationships between the assemblages and environmental data for the fall indicated that depth and bottom salinity were significantly related to the juvenile assemblages and cross-shelf station groups (Table 6).

The inclusion of environmental variables in the analysis of winter juvenile assemblage showed a weak relationship with the cross-shelf groups and juvenile assemblages, as indicated by the CCA correlation (Table 5). The addition of the environmental data also changed the ordination of the stations (Fig. 5D); yet there was still a cross-shelf gradient. Along the first dimension, depth, bottom temperature, and stratification were related to the juvenile assemblages and cross-shelf groups (Fig. 5D). The shallow inner- and mid-shelf groups were lower in bottom temperature and stratification, whereas bottom density was lowest on the inner- and outer-shelves and highest on the mid-shelf (Fig. 2). A Monte Carlo permutation test for the winter relationships indicated that depth and bottom density were significantly correlated with the fish assemblages and station groups (Table 6), although the bottom density did not appear to have any relation to the patterns described (Fig. 5D).

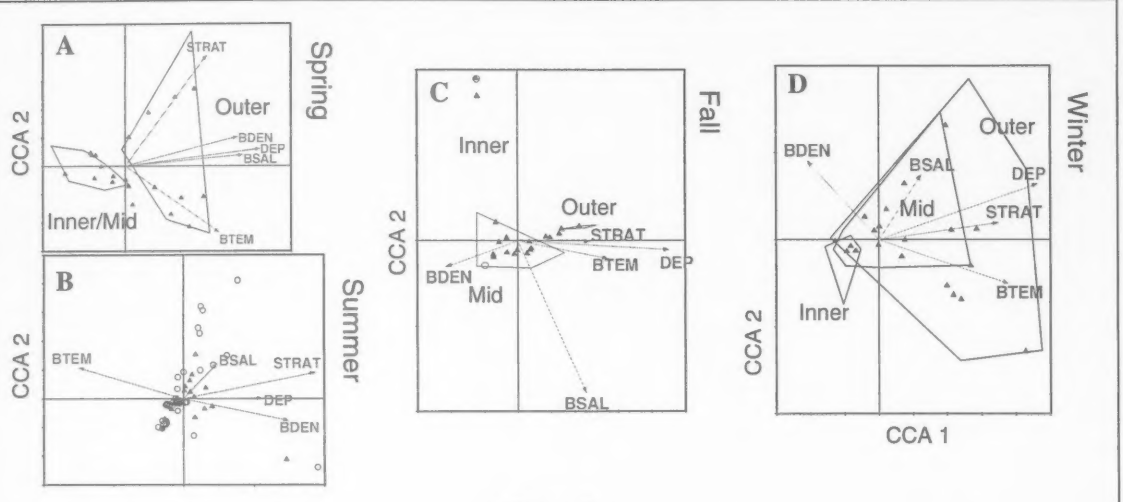

Figure 5

Canonical correspondence analysis ordinations (portraying the first and second dimension scores) of the 10% cross-shelf data set showing the correlations between environmental variables, species, and station groups each season; spring (**A**), summer (**B**), fall (**C**), and winter (**D**). The solid triangles mark the locations of taxa, and the polygons enclose the boundary of each station group with three or more stations (as in Fig. 3). If stations were not grouped, circles mark the locations. The arrows depict the gradient of each environmental variable (temperature=btemp, salinity=bsal, density=bden, stratification=strat, and depth=dep). The dashed lines intersect at the origin of the plot. Analyses were conducted by using both juvenile abundance and environmental data.

Seasonal patterns in juvenile fish assemblages

Many of the species (>68%) were present on the shelf in more than one season, which resulted in a large overlap of the seasonal assemblages (ordination not shown), including 12 of the 23 reef-associated species (Table 2). The assemblage data could be explained in three dimensions (as determined by CA of the 10% cross-shelf data set [Table 5]) and portrayed seasonal and cross-shelf patterns. Along the first dimension, there was separation between the winter inner-shelf assemblage and all other assemblages. The second and third dimensions portrayed a cross-shelf gradient in the spring, fall, and winter juvenile assemblage data. The summer assemblage overlapped all the cross-shelf groups from the other seasons except the winter inner-shelf assemblage. High abundances of *Larimus fasciatus* in the fall and *Leiostomus xanthurus* and *Mugil cephalus* in the winter (Table 2) caused these two seasons to be more dissimilar than were spring and summer, which were very similar. Many of the juveniles were found in the same cross-shelf assemblages during each season, whereas others shifted assemblages (Fig. 4).

Relation between seasonal juvenile assemblages and environmental variables

There appeared to be no relationship between the seasonal juvenile assemblages and environmental variables. The products of the CCA eigenvalues and species-environment correlations were extremely low (Table 5), and the CCA ordination of seasonal juvenile assemblages and environmental variables was different from the CA ordination. Thus, over the entire year, environmental variables did not help explain among-season variation in the juvenile assemblage data.

Along-shelf and cross-shelf patterns in the inshore juvenile fish data set

The inshore data set was made up of estuarine, coastal, and open-shelf species (Table 3, Appendix). There was a cross-shelf gradient in the spring and winter (Fig. 6). The innermost station group (8-m) separated from the other station groups along the first dimension (Fig. 7A) in spring. Most taxa from the 8-m assemblage (5 of 7) were not collected in high enough abundance to be included in the cross-shelf data set (Tables 2 and 3). In winter there was also a gradient from shallow to deeper stations (Fig. 6C). The assemblage nearest the origin in the winter (8-m assemblage) included estuarine species that were abundant at all the inshore stations (Table 3, Appendix). The coastal species, however, were more abundant at the deeper (12-m, 15-m, and 18-m) station groups (Table 3). There was no pattern in the along-shelf transects, from north to south, during either season (Fig. 6, B and D).

Along-shelf and cross-shelf patterns in the offshore juvenile fish data set

The offshore juvenile fish data set included primarily (76%) coastal, open-shelf, and slope species (Table 4, Appendix) and patterns in the data set varied with season. During the fall, there were both cross-shelf (Fig. 7A) and along-shelf patterns (Fig. 7B). Along the first dimension, there was a cross-shelf gradient (Fig. 7A) from the shallow stations (30–45 m depth; Fig. 1C) to the 50-m station group. The juvenile assemblage at the 50-m station group had high abundances of reef-associated species (Table 4). The stations on the northern most transect (Fig. 1C) also separated from the others along the second dimension, and had higher abundances of reef-associated species. The cross-shelf pattern was present in the winter, with greater abundances of reef-associated species at the 50-m station group (Fig. 7C), but there was no along-shelf pattern (Fig. 7D).

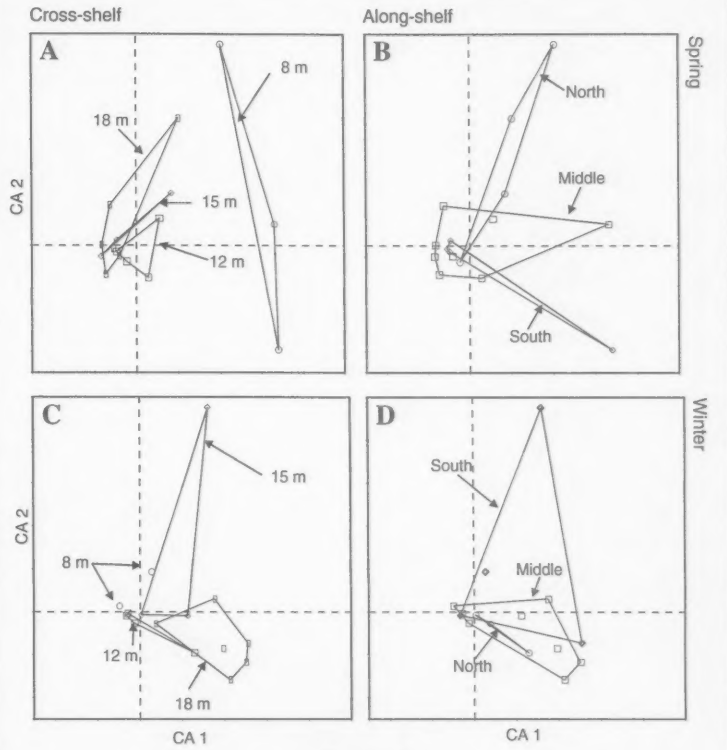

Figure 6

Correspondence analysis ordinations (portraying the first and second dimension scores) of the inshore juvenile fish community data showing cross-shelf and along-shelf station groups in spring (**A** and **B**) and winter (**C** and **D**). Solid lines enclose the boundary of each station group with three or more stations. Station groups comprising one or two stations are not enclosed by a solid line. Each station group is labeled and portrayed with a different symbol. The dashed lines intersect at the origin of the plot. Analyses were conducted by using juvenile fish abundance data only.

Discussion

One hundred and twenty-one taxa of juvenile fish were collected on unconsolidated sediments on the continental shelf off Georgia. The actual number of species was higher, but taxonomic problems limited species identification (e.g., identification of *Etropus* spp., *Prionotus* spp., *Sphoeroides* spp., and *Microgobius* spp.). With concurrent ichthyoplankton sampling of the cross-shelf stations (Fig. 1), we collected 161 taxa (Marancik et al., 2005); the larval community had more pelagic species, including several scombrids (e.g., *Auxis rochei, Scomberomorus cavalla,* and *S. maculatus*) and 17 myctophids. The large juvenile and adult fish communities of the shallow coastal zone (<10 m) and continental shelf (9–180 m) comprised about 150 species, including both pelagic and demersal species (Wenner et al.[4,5,6,7]; Wenner and Sedberry, 1989). A maximum of 164 species were reported from rocky-reefs in the region (Chester et al., 1984; Parker et al. 1994; Baron et al., 2004). Fewer species inhabit estuaries than shelf habitats of the southeast (<90), particularly

[4] Wenner, C. A., C. A. Barans, B. W. Stender, and F. H. Berry. 1979a. Results of MARMAP otter trawl investigations in the South Atlantic Bight. II, spring 1974, 78 p. [Available from Marine Resources Division, South Carolina Department of Natural Resources, 217 Ft. Johnson Rd., P.O. Box 12559, Charleston, SC 29412.]

[5] Wenner, C. A., C. A. Barans, B. W. Stender, and F. H. Berry. 1979b. Results of MARMAP otter trawl investigations in the South Atlantic Bight. III, summer 1974, 62 p. [Available from Marine Resources Division, South Carolina Department of Natural Resources, 217 Ft. Johnson Rd., P.O. Box 12559, Charleston, SC 29412.]

[6] Wenner, C. A., C. A. Barans, B. W. Stender, and F. H. Berry. 1979c. Results of MARMAP otter trawl investigations in the South Atlantic Bight. IV, winter-early spring 1975, 59 p. [Available from Marine Resources Division, South Carolina Department of Natural Resources, 217 Ft. Johnson Rd., P.O. Box 12559, Charleston, SC 29412.]

[7] Wenner, C. A., C. A. Barans, B. W. Stender, and F. H. Berry. 1980. Results of MARMAP otter trawl investigations in the South Atlantic Bight. V, summer 1975, 57 p. [Available from Marine Resources Division, South Carolina Department of Natural Resources, 217 Ft. Johnson Rd., P.O. Box 12559, Charleston, SC 29412.]

if different habitat types are considered (Ross and Epperly, 1985; Hettler, 1989; Nelson et al., 1991). Thus, unconsolidated sediments are as rich a habitat in terms of the number of species, as are pelagic, rocky reef, and estuarine habitats in the southeast U.S. continental shelf ecosystem.

Cross-shelf regions were defined on the Georgia shelf from spatial patterns in juvenile fish distributions. The number and extent of cross-shelf regions varied seasonally, but in general, three regions were identified: inner-shelf, mid-shelf, and outer-shelf regions. Juvenile assemblages were associated with the regions, yet some individual species were distributed across regions. Juveniles of year-round residents (e.g., *Ophidion selenops, Diplectrum formosum,* and *Prionotus carolinus*) were usually the most abundant species, although transient juveniles (e.g., *Leiostomus xanthurus, Lagodon rhomboides*, and *Brevoortia tyrannus*) were seasonally abundant. Cross-shelf gradients in species distribution have been found for other organisms and life stages of fish on the southeast U.S. shelf (macroinfauna: Atkinson et al., 1985; larval fish: Marancik et al., 2005; adult reef fish: Chester et al., 1984; adult demersal fish: Wenner et al.[4,5,6,7]). Cross-shelf gradients also are common in demersal juvenile fish distributions in other continental shelf ecosystems (northwest U.S.: Norcross et al., 1997; Toole et al., 1997; Abookire and Norcross, 1998; Bailey et al., 2003; Johnson et al., 2003; northeast U.S.: Steves et al., 2000; Sullivan et al., 2000; southwest U.S.: Johnson et al., 2001).

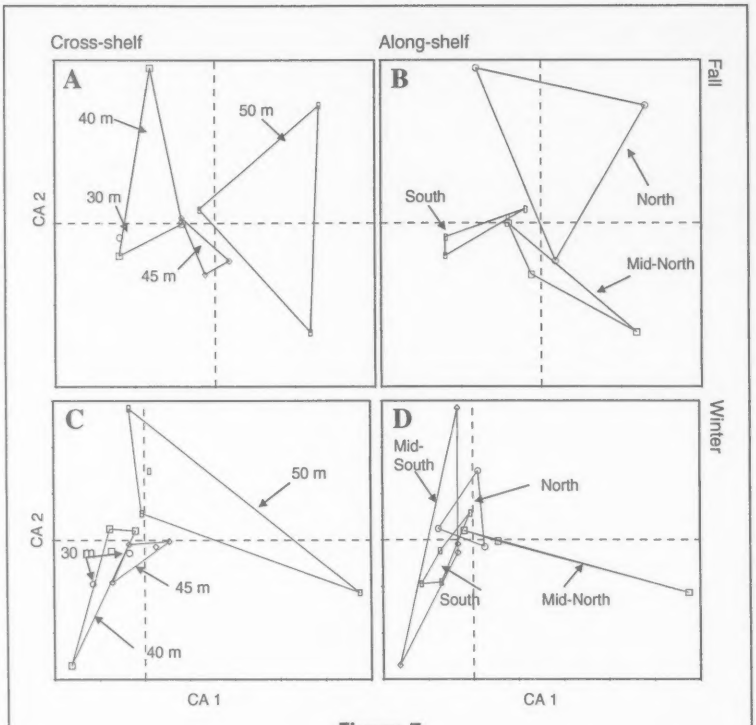

Figure 7

Correspondence analysis ordinations (portraying the first and second dimension scores) of the offshore juvenile fish community data showing cross-shelf and along-shelf station groups in fall (**A** and **B**) and winter (**C** and **D**). Solid lines enclose the boundary of each station group with three or more stations. Station groups comprising one or two stations are not enclosed by a solid line. Each station group is labeled and portrayed with a different symbol. The dashed lines intersect at the origin of the plot. Analyses were conducted by using juvenile fish abundance data only.

The cause of cross-shelf gradients in juvenile fish distribution is difficult to determine. The primary environmental variable correlated with cross-shelf juvenile fish assemblages is often depth (Table 6; Norcross et al., 1997; Steves et al., 2000; Sullivan et al., 2000; Johnson et al., 2001; Johnson et al., 2003), although temperature, salinity, and sediment grain size also are correlated with depth (Norcross et al., 1997; Steves et al., 2000; Sullivan et al., 2000). On the Georgia shelf, salinity, density, and stratification correlated with the distribution of juvenile assemblages during the spring, fall, and winter (Table 6), and along with temperature (Figs. 2 and 6) likely influenced the distribution of juvenile fish, but the causative mechanisms remain unresolved. On the northeast U.S. shelf, cold bottom water left from winter resides on the mid-shelf during summer and fall (cold pool; Ketchum and Corwin, 1964). Steves et al. (2000) and Sullivan et al. (2000) hypothesized that cross-shelf patterns in settlement and juvenile fish distributions were caused by cross-shelf temperature gradients related to the presence of the cold pool on the mid-shelf. Similarly, juvenile flatfish species in Alaska exhibited cross-shelf gradients in habitat use that were influenced by temperature-depth interactions (Norcross et al., 1997). *Hippoglossoides elassodon* were most abundant in the colder, deeper locations, and *Hippoglossus stenolepis* were more abundant in the warmer, shallow locations (Norcross et al., 1997). On the Georgia shelf, juvenile assemblages were less distinct during summer, when environmental gradients were weakest, providing some support for the hypothesis that cross-shelf patterns in juvenile distribution were caused by environmental factors. Alternatively, in other studies it has been hypothesized that juvenile distribution results from selection of specific habitat characteristics within large-scale environmental gradients (Stoner and Abookire, 2002). For example, laboratory and field

studies have found that some fish species select specific sediment characteristics or biogenic structures (Stoner and Abookire, 2002; Diaz et al., 2003; Stoner and Titgen, 2003), and spatial patterns in these habitat characteristics may cause spatial patterns in juvenile fish distribution. We did not stratify sampling by sediment characteristics or biogenic structures and thus we could draw no conclusion regarding the role of habitat characteristics in influencing juvenile fish distribution on the Georgia shelf.

No along-shelf spatial patterns were found in the juvenile fish community on the continental shelf off the Georgia coast; however, the along-shelf dimension sampled (~60 km) was relatively small compared to the along-shelf dimension of the southeast U.S. continental shelf ecosystem (~1000 km). Consistent patterns in along-shelf gradients in oceanographic features (Atkinson et al., 1985; Lee et al., 1991) and adult fish communities (Chester et al., 1984; Wenner and Sedberry, 1989) exist on the southeast U.S. shelf, but over larger along-shelf dimensions than sampled in the present study (200–400 km). Consistent patterns of juvenile use of along-shelf habitat over smaller dimensions (~100 km) have been reported for other shelf ecosystems (northwest U.S.: Norcross et al., 1997; Abookire and Norcross, 1998; northeast U.S.: Steves et al., 2000; Sullivan et al., 2000), but the relation to along-shelf environmental and habitat characteristics is unclear. Along-shelf patterns in juvenile fish distribution on unconsolidated sediments may occur on the southeast U.S. shelf, but at dimensions larger than 60–100 km.

Seasonal patterns in settlement and postsettlement movement both defined and blurred classification of juvenile fish assemblages on the Georgia shelf. Many of the resident shelf species were consistently collected in the same cross-shelf regions (i.e., inner and mid-shelf: *Ophidion selenops, Prionotus carolinus, Dactyloscopus moorei, Stenotomus* sp., and *Serraniculus pumilio*; outer-shelf: *Symphurus minor, Serranus phoebe, Centropristis ocyurus,* and *Bothus lunatus*), and the consistent collection of these species helped form a definition of juvenile assemblages. Other resident species shifted juvenile assemblages (e.g., *Diplectrum formosum, Monacanthus hispidus, Bothus robinsi, Otophidium omostigmum*, and *Bothus ocellatus*), possibly because of seasonal changes in settlement patterns or ontogenetic postsettlement movements, with the result that some classifications were blurred. Four scieanid species, present during the spring, summer, and fall as larvae (Marancik et al., 2005), were collected during the fall as juveniles and contributed to defining the fall inner-shelf (*Larimus fasciatus* and *Menticirrhus americanus*) and mid-shelf (*Cynosion nothus* and *C. regalis*) juvenile assemblages. These species may shift settlement or juvenile habitat (or both) between estuaries and the coastal ocean; this shift has been shown to occur off the coast of New Jersey (Able et al., 2003; Neuman and Able, 2003). Further, a number of species in the ecosystem spawn on the shelf yet use estuarine habitats as juvenile nurseries (Warlen and Burke, 1990; Able and Fahay, 1998). In our study, settlement stage *L. xanthurus, Brevoortia tyrannus*, and *Lagodon rhomboides* were collected on the shelf, and contributed to the definition of the inner- and mid-shelf assemblages. Some individuals of these estuarine-dependent species may not settle directly into estuarine habitats as has been demonstrated for *Centropristis striata* off the coast of New Jersey (Able and Fahay, 1998).

Only a few reef-associated species collected during the study used unconsolidated sediments consistently. *Centropristis ocyurus, Diplectrum formosum*, and *Stenotomus* sp. were the most common reef fishes collected on unconsolidated sediments, and made up >1% of the total catch (Appendix). Other less frequently occurring juveniles of important reef-associated species that were collected were *Centropristis striata, Epinephelus niveatus, Pristigenys alta, Priacanthus arenatus*, and *Lutjanus analis*.

Reef-associated species were collected across the entire shelf. *Centropristis ocyurus* and *Stenotomus* sp. were commonly collected on the outer-, inner-, and mid-shelves, respectively (Table 2, Appendix). *Centropristis striata*, which uses habitats in estuaries and coastal regions as juveniles (Able and Fahay, 1998), was collected on the inner- and mid-shelf in the spring and summer, during periods of high settlement (Able and Hales, 1997). The presence of many reef-associated species at the deeper (50 m) stations (Table 4) may indicate greater use of offshore unconsolidated sediments by reef-associated species or that sampling was done in an area closer to rocky-reefs. Several species of the South Atlantic Fishery Management Council snapper-grouper complex have been reported to use a variety of nonreef habitats as newly settled juveniles, including unconsolidated sediments on the shelf (Lindeman et al., 2000). Many coral reef fish also use several types of nearshore nonreef habitat as juveniles (de la Moriniere et al., 2002; Nagelkerken and van der Velde, 2002). These nonreef habitats are assumed to be important nursery habitats; however, strong evidence of movement from juvenile to adult habitats has been documented only for a few reef-associated fishes (Gillanders et al., 2003). The fact that reef-associated species also use unconsolidated sediments indicates there is an interdependence between reef habitats and unconsolidated sediments; yet the function of each habitat remains unquantified.

In addition to reef species, a number of commercially and recreationally important demersal species used unconsolidated sediments on the shelf off Georgia as juvenile habitat. Juvenile *Cynoscion nothus, C. regalis* and *Menticirrhus americanus* were collected on the inner- and mid-shelves in the fall, and settlement-size *Leiostomus xanthurus* were collected in the winter. However, small noncommercial demersal species were the most abundant juveniles collected during beam trawl sampling.

Much of the southeast U.S. continental shelf is impacted by trawl fisheries that can adversely impact unconsolidated sediments (Barnette[3]). Current manage-

ment concerns focus primarily on bycatch reduction, impacts on stock assessments of commercially and recreationally important finfish, and destruction of coral, live hard-bottom, and SAV habitat (NOAA[8]; Barnette[3]). The presence of reef-associated and sciaenid species in our samples indicate that the unconsolidated sediments of the shelf are potentially important habitats for early life stages, and that trawl fisheries on the shelf may impact the population dynamics of these species. Equally important may be the ecological effects on the small noncommercial demersal species that dominate the catch on unconsolidated sediments and that are abundant in the shrimp trawl bycatch (SAFMC, 1996).

Unconsolidated sediments serve as juvenile habitat for a number of species in the southeast U.S. shelf ecosystem. Some species use unconsolidated sediments throughout their life history, but for a number of species, unconsolidated sediments serve as only one of a mosaic of habitats through the life history (see Browman and Konstantinos, 2004). Additionally, there is growing recognition of the importance of unconsolidated sediments in the trophic ecology of the southeast U.S. shelf. Benthic primary production of the shelf has been shown to be 4–6 times greater than water-column production at specific locations (Nelson et al., 1999). Unconsolidated sediments are areas of reef fish feeding (Sedberry, 1985, 1990) and several important fish predators inhabit unconsolidated sediments (triglids, synodontids, Ross, 1976; Richards et al., 1979). Current ecosystem approaches to fisheries (see Sissenwine and Murawski, 2004) in the region largely ignore unconsolidated sediments (SAFMC, 1998, 2001), and although the importance of this habitat to juvenile fish production remains unquantified (sensu Beck et al., 2001), management efforts would be improved through a greater recognition of the potential importance of these habitats to fisheries production and the interconnectedness of multiple habitats in the southeast U.S. continental shelf ecosystem.

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Appendix

Juvenile taxa collected in beam trawl collections from the continental shelf off the coast of Georgia, USA. Seasonal catch per unit of effort (CPUE, fish/5 min) was calculated from cross-shelf stations (see Fig. 1 and Table 1). Mean (SD) of the total CPUE and standard length (SL, mm) was calculated from all stations. Adult distributions (AD) and whether species are reef-associated (RA) was determined from the literature. E = estuarine, C = coastal (0–20 m), O = open-shelf (20–70 m), Sl = slope (>70 m), W = weakly reef-associated, and S = strongly reef-associated.

		Cross-shelf stations				All stations			
		Mean				CPUE	SL (mm)		
Family	Species	Spring	Summer	Fall	Winter	Mean (SD)	Mean (SD)	AD	RA
Elopidae	*Elops saurus*		0.02		0.01	0.007 (±0.084)	46.7 (±15.9)	E, C	
Muraenidae	*Gymnothorax saxicola*					0.005 (±0.069)	69.5 (±0.7)	C, O	
Ophichthidae	*Ophichthus gomesii*		0.01			0.009 (±0.119)	103.3 (±20.1)	E, C, O	
	Ophichthus ocellatus	0.05		0.03	0.02	0.016 (±0.133)	160.9 (±59.2)	E, C, O	
Congridae	*Ariosoma balearicum*	0.39	0.10	0.30	0.02	0.135 (±0.431)	126.5 (±44.2)	E, C, O	
Clupeidae	*Brevoortia tyrannus*				0.07	0.014 (±0.116)	24.5 (±1.8)	E, C, O	
	Etrumeus teres	0.02			0.01	0.005 (±0.069)	31.8 (±1.1)	C, O	
	Sardinella aurita		0.01			0.005 (±0.069)	36.0 (±2.8)	C, O	
Engraulididae	*Anchoa hepsetus*	0.09	0.19	1.14		0.355 (±2.631)	31.3 (±10.2)	E, C, O	
	Anchoa lamprotaena			0.07		0.015 (±0.215)	44.5 (±5.8)	C	
Argentinidae	*Argentina striata*				0.02	0.005 (±0.069)	52.0 (±1.4)	Sl	
Synodontidae	*Synodus foetens*	1.00	0.45	1.55	0.39	0.643 (±1.570)	65.8 (±40.1)	E, C, O	
	Synodus poeyi	0.26				0.035 (±0.420)	33.1 (±3.5)	O	
	Trachinocephalus myops	0.13	0.06	0.22	0.06	0.091 (±0.354)	92.3 (±47.6)	O	
Gadidae	*Urophycis regia*	0.28			0.68	0.302 (±0.867)	69.7 (±21.3)	C, O	
Ophidiidae	*Ophidion grayi*	0.02				0.002 (±0.049)	39.0	C, O	
	Ophidion holbrooki	0.70				0.121 (±1.226)	98.8 (±47.3)	C, O	
	Ophidion marginatum	0.11	0.03	0.01	0.03	0.050 (±0.270)	94.8 (±43.0)	C, O	
	Ophidion selenops	3.63	0.35	4.92	1.49	1.823 (±4.373)	56.1 (±19.7)	O	
	Ophidion welshi	0.12		0.02		0.019 (±0.194)	107.9 (±27.1)	C, O	
	Otophidium omostigmum	0.60	0.09	0.69	0.38	0.392 (±1.192)	60.0 (±21.8)	O	
Batrachoididae	*Porichtys plectrodon*			0.03		0.007 (±0.078)	56.3 (±13.7)	C, O	
Antennariidae	*Antennarius ocellatus*					0.002 (±0.048)	47.0	O, Sl	W
	Antennarius radiosus				0.02	0.010 (±0.102)	24.0 (±4.1)	O, Sl	W
Ogcocephalidae	*Halieutichthys aculeatus*	0.02	0.04			0.014 (±0.118)	14.4 (±1.5)	C, O	
	Ogcocephalus nasutus	0.02	0.05		0.01	0.025 (±0.154)	29.0 (±17.8)	C, O	
	Ogcocephalus parvus				0.02	0.012 (±0.108)	75.6 (±34.1)	O	

continued

Appendix (continued)

		Cross-shelf stations				All stations			
		Mean				CPUE	SL (mm)		
Family	Species	Spring	Summer	Fall	Winter	Mean (SD)	Mean (SD)	AD	RA
Exocetidae	*Hemiramphus balao*			0.02		0.003 (±0.052)	80.0	C, O	
	Hemiramphus brasilieusis		0.01			0.002 (±0.049)	97.0	C, O	
	Hirundichthys affinis		0.05	0.02		0.017 (±0.146)	35.4 (±13.8)	C, O	
	Parexocoetus brachypterus			0.02		0.002 (±0.048)	34.0	O	
Syngnathidae	*Hippocampus erectus*	0.12	0.04	0.01	0.04	0.055 (±0.236)	60.8 (±19.9)	E, C	
	Syngnathus scovelli		0.06	0.02		0.019 (±0.166)	56.3 (±11.8)	E, C	
	Syngnathus springeri	0.02	0.01		0.07	0.021 (±0.144)	64.3 (±6.9)	C, O	
Scorpaenidae	*Scorpaena dispar*		0.02	0.07		0.043 (±0.458)	39.4 (±21.6)	C, O	W
	Scorpaena plumieri	0.07	0.13	0.08	0.02	0.078 (±0.335)	43.5 (±31.0)	C, O	W
Triglidae	*Prionotus* spp.	0.73	0.17	15.31	0.52	2.700 (±9.684)	11.6 (±3.3)	C, O	
	Bellator brachychir		0.01	0.01		0.007 (±0.079)	27.0 (±13.2)	O	
	Bellator militaris	0.01	0.01			0.004 (±0.063)	17.5 (±12.0)	O	
	Prionotus carolinus	3.04	0.32	0.42	1.08	1.127 (±2.801)	53.5 (±27.9)	E, C, O	
	Prionotus evolans	0.03				0.004 (±0.081)	90.0 (±62.2)	E, C, O	
	Prionotus ophryas		0.01			0.002 (±0.049)	16.0	C, O	
	Prionotus scitulus				0.08	0.033 (±0.180)	133.3 (±19.4)	C, O	
Serranidae	*Centropristis ocyurus*	0.73	0.04		0.08	0.313 (±1.592)	31.0 (±12.0)	O	W
	Centropristis striata	0.07	0.04			0.017 (±0.161)	33.1 (±14.8)	E, C, O	S
	Diplectrum formosum	0.96	2.50	2.39	0.16	1.360 (±3.378)	28.2 (±23.8)	C, O	W
	Epinephelus niveatus					0.005 (±0.069)	29.0 (±7.1)	Sl	S
	Serraniculus pumilio	0.03	0.21	0.34	0.12	0.155 (±0.734)	22.5 (±7.5)	C, O	W
	Serranus phoebe	0.31	0.04	0.02	0.04	0.220 (±1.618)	21.6 (18.1)	C, O	W
	Serranus subligarius					0.007 (±0.146)	30.7 (±9.3)	C, O	S
Grammistidae	*Rypticus* spp.			0.01		0.046 (±0.590)	41.8 (±12.9)	C, O	S
	Rypticus bistrispinus					0.054 (±0.596)	42.5 (±5.2)	C, O	S
Priacanthidae	*Priacanthus arenatus*		0.01			0.002 (±0.049)	15.0	O	S
	Pristigenys alta		0.02	0.05		0.016 (±0.126)	17.7 (±8.6)	O	S
Apogonidae	*Apogon* sp.	0.01	0.03			0.011 (±0.125)	20.9 (±11.0)	O	W
	Astrapogon spp./*Epigonus* spp.		0.01			0.002 (±0.049)	9.2		
	Apogon maculatus					0.061 (±0.680)	17.6 (±3.5)	O	W
	Apogon pseudomaculatus		0.04			0.028 (±0.213)	18.6 (±14.1)	O	W
Carangidae	Carangidae				0.02	0.005 (±0.068)	18.5 (±6.4)		
	Decapterus spp.		0.14			0.031 (±0.278)	24.8 (±5.7)	O	
	Caranx bartholomaei	0.02				0.002 (±0.044)	12.0	C, O	
	Caranx crysos			0.02		0.002 (±0.049)	101.0	C, O	W
	Chloroscombrus chrysurus		0.01	0.06		0.010 (±0.107)	15.1 (±3.6)	C, O	W
	Decapterus macarellus	0.02			0.01	0.005 (±0.069)	23.0 (±4.2)	O	
	Decapterus punctatus		0.05	0.01		0.017 (±0.188)	27.6 (±5.8)	C, O	
	Trachurus lathami	0.02				0.002 (±0.046)	16.0	C, O	
Lutjanidae	*Lutjanus analis*		0.01			0.002 (±0.049)	18.0	C, O	S
Haemulidae	*Haemulon aurolineatum*		0.05	0.05		0.016 (±0.185)	45.3 (±26.8)	C, O	W
Sparidae	*Stenotomus* sp.	0.18	0.16	0.14	0.08	0.135 (±0.823)	46.1 (±28.4)	C, O	W
	Lagodon rhomboides				0.06	0.012 (±0.128)	27.1 (±27.9)	E, C, O	W
Sciaenidae	*Cynoscion nothus*		0.04			0.006 (±0.095)	9.8 (±0.9)	E, C	
	Cynoscion regalis		0.06			0.008 (±0.102)	10.3 (±0.8)	E, C	
	Larimus fasciatus		0.19			0.027 (±0.313)	14.7 (±4.7)	E, C	
	Leiostomus xanthurus			2.27		3.361 (22.193)	16.2 (±1.7)	E, C, O	

continued

Appendix (continued)

		Cross-shelf stations				All stations			
		Mean				CPUE	SL (mm)		
Family	Species	Spring	Summer	Fall	Winter	Mean (SD)	Mean (SD)	AD	RA
Sciaenidae (cont.)	*Menticirrhus americanus*		0.07			0.010 (±0.159)	13.3 (±9.1)	E, C	
	Stellifer laceolatus					0.005 (±0.097)	75.0 (±0.0)	E, C	
Mullidae	*Upeneus parvus*					0.002 (±0.049)	48.0	C, O	
Mugilidae	*Mugil cephalus*				0.18	0.047 (±0.422)	21.7 (±2.3)	E, C, O	
	Mugil curema		0.01		0.02	0.027 (±0.214)	21.8 (±1.5)	E, C, O	
Labridae	*Halichoeres bivittatus*		0.02			0.010 (±0.152)	71.0 (±5.0)	O	S
	Xyrichtys novacula		0.01			0.002 (±0.049)	41.5	O	
Scaridae	Scaridae		0.03			0.009 (±0.093)	41.3 (±31.8)		
Uranoscopidae	*Kathetostoma albigutta*				0.01	0.002 (±0.049)	26.0	O	
Dactyloscopidae	*Dactyloscopus moorei*	0.80	0.17	2.09	0.80	0.689 (±2.073)	22.1 (±5.1)	O	
Blenniidae	*Chasmodes/Parablennius marmoreus*		0.04			0.007 (±0.109)	19.0 (±2.6)	E, C	S
	Hypleurochilus geminatus	0.04	0.01			0.021 (±0.160)	43.6 (±17.2)	E, C	
	Parablennius marmoreus		0.02			0.009 (±0.119)	20.3 (±4.3)	E, C	S
Callionymidae	*Diplogrammus pauciradiatus*		0.07			0.025 (±0.170)	9.5 (±1.0)	O	
Gobiidae	*Microgobius* spp.	0.06	0.82	1.42	0.01	0.454 (±1.424)	16.7 (±1.9)		
	Gobiosoma bosci				0.01	0.014 (±0.150)	19.5 (±1.5)	E, C	
	Gobiosoma ginsburgi				0.02	0.005 (±0.068)	22.5 (±3.5)	E, C	W
	Ioglossus calliurus	0.01				0.002 (±0.041)	14.7	C, O	
Stromateidae	*Peprilus triacanthus*	0.07	0.01		0.08	0.042 (±0.250)	25.3 (±11.7)	C, O	
Bothidae	*Bothus ocellatus/robinsi*	0.07	0.08	0.07		0.041 (±0.254)	15.9 (±2.6)		
	Bothus spp.	0.86	0.41	4.50	0.33	0.971 (±3.728)	18.4 (±3.0)	O	
	Bothus lunatus	0.02			0.01	0.005 (±0.069)	79.0 (±9.9)	E, C, O	
	Bothus ocellatus		0.03	0.04	0.01	0.046 (±0.341)	33.4 (±23.5)	C, O	
	Bothus robinsi	1.21	0.23	0.52	0.34	0.435 (±1.464)	37.3 (±35.7)	C, O	
Paralichthyidae	*Citharichthys* spp.	0.19	0.03		0.41	0.132 (±0.671)	13.7 (±6.0)		
	Etropus spp.	8.89	1.31	2.96	15.97	10.563 (20.936)	18.3 (±8.2)		
	Ancylopsetta quadrocellata				0.22	0.114 (±0.386)	38.1 (±17.7)	C, O	
	Citharichthys gymnorhinus	0.03				0.004 (±0.063)	18.5 (±7.8)	O	
	Citharichthys macrops	0.20	0.04	0.02	0.13	0.090 (±0.441)	76.9 (±44.8)	C, O	
	Cyclopsetta fimbriata	0.03	0.04	0.05		0.037 (±0.226)	28.9 (±14.5)	O	
	Syacium papillosum	0.02	0.09	0.04		0.040 (±0.269)	16.5 (±17.2)	C, O	
Scopthalmidae	*Scopthalmus aquosus*				0.01	0.002 (±0.049)	14.0	E, C, O	
Soleidae	*Gymnachirus melas*	0.04	0.03			0.014 (±0.115)	17.1 (±7.5)	O	
Cynoglossidae	*Symphurus* spp.		0.01	0.02		0.005 (±0.067)	18.0 (±9.9)		
	Symphurus diomedeanus	0.05				0.007 (±0.109)	22.5 (±17.7)	O	
	Symphurus minor	0.26	0.25	1.87	0.15	0.552 (±1.878)	23.8 (±4.2)	O	
	Symphurus parvus	0.05	0.02			0.012 (±0.108)	15.9 (±1.7)	O	
	Symphurus plagiusa		0.02			0.029 (±0.270)	59.9 (±16.5)	E, C, O	
	Symphurus urospilus	0.42	0.24	0.43	0.28	0.257 (±0.717)	55.5 (±20.8)	O	
Balistidae	*Aluterus schoepfi*		0.01	0.02		0.005 (±0.066)	18.0 (±11.3)	C, O	W
	Cantherhines pullus					0.012 (±0.128)	18.5 (±1.5)	S	
	Monacanthus ciliatus	0.02				0.005 (±0.069)	41.0 (±26.9)		
	Monacanthus hispidus	0.18	0.54	1.36	0.06	0.460 (±1.314)	34.4 (±22.6)	C, O	
	Monacanthus setifer			0.01		0.004 (±0.063)	91.0 (±0.0)	O	
Ostraciidae	*Lactophyrs quadricornis*		0.01			0.009 (±0.090)	67.8 (±83.6)	O	W
Tetraodontidae	*Sphoeroides* spp.	0.03	0.13		0.01	0.126 (±0.600)	15.9 (±4.0)		
Diodontidae	*Chilomycterus schoepfi*	0.02				0.002 (±0.049)	17.0	E, C	

Growth and maturity of salmon sharks (*Lamna ditropis*) in the eastern and western North Pacific, and comments on back-calculation methods

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Abstract—Age and growth estimates for salmon sharks (*Lamna ditropis*) in the eastern North Pacific were derived from 182 vertebral centra collected from sharks ranging in length from 62.2 to 213.4 cm precaudal length (PCL) and compared to previously published age and growth data for salmon sharks in the western North Pacific. Eastern North Pacific female and male salmon sharks were aged up to 20 and 17 years, respectively. Relative marginal increment (RMI) analysis showed that postnatal rings form annually between January and March. Von Bertalanffy growth parameters derived from vertebral length-at-age data are L_∞=207.4 cm PCL, k=0.17/yr, and t_0=−2.3 years for females (n=166), and L_∞=182.8 cm PCL, k=0.23/yr , and t_0=−1.9 years for males (n=16). Age at maturity was estimated to range from six to nine years for females (median precaudal length of 164.7 cm PCL) and from three to five years old for males (median precaudal length of 124.0 cm PCL). Weight-length relationships for females and males in the eastern North Pacific are $W=8.2\times10^{-05}\times L^{2.759}$ (r^2=0.99) and $W=3.2\times10^{-06}\times L^{3.383}$ (r^2=0.99), respectively. Our results show that female and male salmon sharks in the eastern North Pacific possess a faster growth rate, reach sexual maturity earlier, and attain greater weight-at-length than their same-sex counterparts living in the western North Pacific.

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The salmon shark (*Lamna ditropis*) is a large apex predator inhabiting the coastal and oceanic waters of the North Pacific Ocean, most commonly ranging from 65°N latitude to 35°N in the west and to 30°N in the east (Strasburg, 1958; Neave and Hanavan, 1960; Compagno, 1984, 2001; Blagoderov, 1994; Nagasawa, 1998). It is found individually and in large aggregations at sea-surface temperatures of 5°C to 18°C and has a depth distribution ranging from the surface to at least 150 m (Compagno, 1984, 2001). The salmon shark is a highly opportunistic predator, feeding on a wide variety of prey and sharing the highest trophic level of the food web in boreal and temperate North Pacific waters with marine mammals and seabirds (Brodeur, 1988; Nagasawa, 1998). Adult salmon sharks typically range in size from 180–210 cm precaudal length (PCL; = 200–260 cm total length, TL) and can weigh up to 220 kg (Tanaka, 1980; JAMARC, 1980; Nagasawa, 1998). Reported lengths of 300 cm TL and greater and weights exceeding 450 kg are unsubstantiated.

Salmon sharks are migratory in nature. North-south migrations have been documented on both sides of the North Pacific; northern movements occur in spring and southern movements are occur in autumn (Iino, 1939; Kosugi and Tsuchisaki, 1950; Gorbatenko and Cheblukova, 1990; Balgaderov, 1994; Nakano and Nagasawa, 1994). However, this species is present in boreal waters throughout the year (Goldman and Human, in press; Weng et al., 2005). Salmon sharks tagged with satellite transmitters have shown an extremely wide range of movements throughout the eastern North Pacific—movement as far south as southern California and Hawaii (Weng et al., 2005). To date, however, no salmon shark has been recorded moving across the Pacific, but such movements are suspected to occur (Nakano and Nagasawa 1996; Goldman and Musick, in press; Weng et al., 2005)

Sexual segregation is relatively common in sharks; however an extremely large sex ratio difference exists in salmon sharks across the North Pacific basin (Sano, 1962; Nagasawa, 1998; Goldman and Musick, in press). The western North Pacific (WNP) is male dominated and the eastern North Pacific (ENP) is female dominated. Male dominance in the WNP and female dominance in the ENP increase with increasing latitude. Larger sharks range farther north than smaller individuals, and

southern catches generally occur in deeper waters (Nagasawa, 1998; Goldman and Musick[1]).

Shark catches in Alaska waters have been reported to be as high as those from Washington, Oregon and California combined (Camhi, 1999), and there is concern over the amount of shark bycatch being taken (Gaichas[2]). In 1997, state managers closed commercial shark fishing in Alaska state waters and imposed conservative sport-fishing limits (1 shark per person per day, 2 per year) that legally encompass federal waters too. Federal managers are currently addressing elasmobranch management issues (Goldman, 2001).

Because of the ever-increasing importance of providing accurate and timely life history parameters to foster responsible management efforts, we had two main objectives in our study. The first was to estimate growth parameters and the age and length at sexual maturity of salmon sharks in the ENP. The second was to compare our results to those previously published on salmon sharks from the WNP to elucidate any existing variability in the life history parameters of this highly sexually segregated population. Lastly, because of the small number of samples for female juvenile age classes, a small overall sample size for males, and the fact that samples (for both sexes) were not obtained for each month, we applied several back-calculation methods to our data (see Cailliet and Goldman, 2004 and Goldman, 2004) and provide a demonstration and brief discussion on the importance of choosing an appropriate back-calculation method.

Materials and methods

Salmon shark vertebrae were obtained from numerous sources and locations ranging geographically from southern California to Kodiak Island (n=182) between 1997 and 2002. The majority of samples came from research cruises in the Gulf of Alaska (GOA) and Prince William Sound (PWS), by accompanying sport-fishing vessels on salmon shark trips in the GOA and PWS, and from Alaska Department of Fish and Game (ADF&G) port samplers. Vertebral samples collected from British Columbia to southern California came from incidental catches and recently beached animals.

Precaudal, fork, and total length (PCL, FL, and TL) were measured on a straight line and recorded along with date, location, and sex (and clasper length in males). Samples provided by additional sources included at least one of the necessary straight-line measurements, date and location of capture or wash-up, and sex. Weights were obtained from 20 females ranging from 67.1 to 213.4 cm PCL, and 7 males ranging from 69.5 to 187 cm PCL. The power function, $W = aL^b$, was fitted to the data (in SigmaPlot, vers. 6.0, SPSS Inc., Chicago, IL), where W = weight (kg) and L = length (cm PCL). Specimens weighed had been collected from southern California to Alaska. A likelihood ratio test was used to determine whether differences between female and male weight-length parameters were significant or if a single set of parameters described the data better (Kimura, 1980; Quinn and Deriso, 1999; Haddon, 2001) (SAS, vers. 8.0, SAS, Cary, NC). A 20- to 25-cm section of vertebrae was removed from directly in front of the first dorsal fin (above the gills) and stored frozen. We used PCL measurements to make direct comparisons with previously published salmon shark age and growth data from the WNP. Linear regression equations, based on our samples, were developed for converting PCL to FL and TL.

Vertebral samples were thawed, cleaned of excess tissue, separated into individual centra and stored in 70% ethyl alcohol for at least 24 h. Centra were sagittally sectioned immediately adjacent to the center of the focus and then cut again approximately 1.5 mm off-center by using an Isomet rotary diamond saw (Buehler, Lake Bluff, IL). The sections were pressed between two pieces of Plexiglas (to prevent warping), air-dried for 24 h under a ventilation hood, and then mounted onto microscope slides with a mounting medium. After drying, sections were polished with wet fine grit (320-, 400-, and 600-grit) sand paper to approximately 0.5 mm and air-dried. Sections were viewed by using a binocular dissecting microscope with transmitted light.

Centrum radius (CR) and distance to the outer margin of each ring were measured to the nearest 0.001 mm along a straight line from the central focus to the center of the outer margin of the corpus calcareum (Fig. 1A) using a compound video microscope with the Optimus image analysis system (Media Cybernetics, vers. 6.5., Silver Spring, MD). PCL was plotted against CR to determine the proportional relationship between somatic and vertebral growth.

A banding pattern was readily distinguishable in sectioned centra; wide bands were separated by distinct narrow opaque bands. This pattern occurred on both arms of the corpus calcareum and extended across the intermedialia. Each pair of wide-narrow bands was considered a growth cycle; the narrow opaque bands, hereafter referred to as "rings," were counted (Fig. 1A). An angle change in the intermedialia and a ring on the corpus calcareum were present approximately 5 to 6 mm from the focus of each centrum and were considered to represent a birthmark. The "prebirth ring" reported by Nagasawa (1998) was present in most specimens just prior to the birthmark but was not counted nor measured (Fig. 1A).[3] A distinct notch was usually

[1] Goldman, K. J., and J. A. Musick. 2002. Unpubl. data. Alaska Department of Fish and Game, 3298 Douglas Pl., Homer, AK 99603, and Virginia Institute of Marine Science, 1208 Greate Rd. Gloucester Pt., VA 23062.

[2] Gaichas, S. 2002. Personal commun. NMFS Alaska Fisheries Science Center, 7600 Sand Point Way NE, Building 4, Seattle WA 98115.

[3] Our aging protocol was independently developed by the senior author and a co-reader, and later found to be identical to that used for salmon sharks in the western North Pacific (H. Nakano, pers. commun.) Vertebral images were exchanged and aged by the senior author and H. Nakano (and read randomly and blindly). The results validated that aging was conducted in an identical manner between studies.

present along the inside edge of the corpus calcareum at each ring, providing an additional aging feature, particularly in sections where the cut excluded the radials of the intermedialia (Fig. 1B). Annulus measurements were made from the origin of a wide band to the outer margin of the adjacent narrow band.

Two readers independently aged all centra two times in blind, randomized trials. This procedure allowed the calculation of within-reader precision, and between-reader precision twice. When there was a disagreement between readers, a final age determination was made by both readers viewing the centrum together. Percent agreement ($PA=[No.\ agreed/No.\ read]\times 100$), and percent agreement plus or minus one year (PA [±1 yr]) were calculated for length groups of 10 cm to test for precision. The criticism of percent agreement as a measure of precision has been that it varies widely among species and ages within a species (Beamish and Fournier, 1981; Campana, 2001). The criticism regarding variation among species is irrelevant because one is only interested in the particular species one is aging (i.e., these particular species are not compared among other species), but the criticism regarding variation of ages within a species is not only relevant, but is true. There is, however, validity in using percent agreement with individuals grouped by length as a test of precision because it does not rely on ages (which have been estimated), but rather on lengths, which are empirical values (Goldman, 2004; Cailliet and Goldman, 2004). Ages could be used if, and only if, all age classes have been validated.

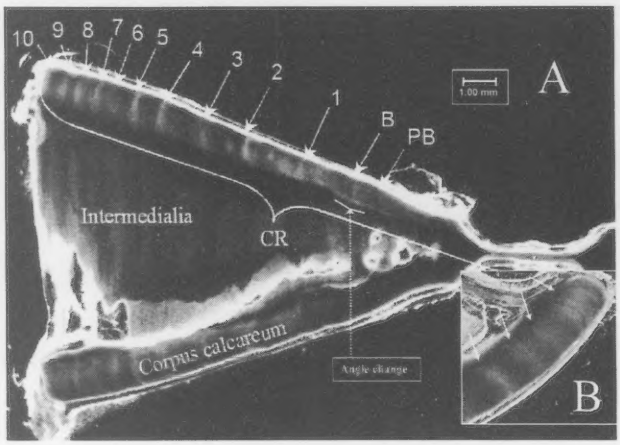

Figure 1

(**A**) Sagittal section of a 10-yr-old salmon shark (*Lamna ditropis*) vertebral centrum showing typical banding pattern. CR=centrum radius. (**B**) Portion of a sagittal section from a salmon shark vertebral centrum without intermedialia, showing the distinct notching pattern (white arrows) that accompanies the banding pattern and that is used to aid in assessing ages. The 1.0-mm bar applies to both A and B. PB = prebirth ring, B = birth ring and numbers indicate rings or age in years.

The most commonly used methods for evaluating precision among age determinations have been the average percent error (APE) technique of Beamish and Fournier (1981) and the modification of their method by Chang (1982). However, Hoenig et al. (1995) have demonstrated (using the Beamish and Fournier, 1981, data) that there can be differences in precision that these methods obscure because with the APE it is assumed that the variability among observations of individual fish can be averaged over all age groups and that this variability can be expressed in relative terms. Also, APE indices do not result in values that are independent of the age estimates, do not test for systematic differences, and do not distinguish all sources of variability (such as differences in precision with age) (Hoenig et al., 1995). Good APE values appear to tell us only which reader was less variable, not which was better or if either was biased, which is more critical in knowing whether reliable ages can be produced and replicated (i.e. is the error within and between readers due to random error or a systematic bias).

Campana et al. (1995) stated the importance of a separate measure for bias, and Hoenig et al. (1995) and Evans and Hoenig (1998) suggested testing for systematic differences between readers using chi-square tests of symmetry to determine whether differences are systematic (biased) or due to random error. These are statistically rigorous and effective methods for detecting bias (Campana, 2001) and were conducted in the present study. These techniques place all age values in contingency tables and test the hypothesis that values in a given table are symmetrical about the main diagonal (Hoenig et al., 1995; Evans and Hoenig, 1998). They can also be set up to test among all individual age classes or groups of age classes (Hoenig et al., 1995). The test statistic (the chi-square variable) will tend to be large if a systematic difference exists between the two readers.

A relative marginal increment (RMI) analysis was used to verify the temporal periodicity of ring formation in the vertebrae. This is a standardized marginal increment analysis whereby the margin, or growth area of a centrum from the last narrow growth ring to the centrum edge, is divided by the width of the last fully formed growth increment (Branstetter and Musick, 1994; Conrath et al., 2002). Resulting RMI values were compared to the month of capture. This analysis was performed on immature and mature sharks separately and combined. Age-zero animals were not included (because they have no fully formed increments).

The von Bertalanffy growth function was fitted to the vertebral age-at-length data for salmon sharks from the ENP with a nonlinear least squares regression algorithm ("nls" in S-Plus, Professional Release 1, Math-

soft Inc., Seattle, WA) to estimate parameters. The von Bertalanffy growth function is

$$L_t = L_\infty\left[1-\exp\left(-k(t-t_0)\right)\right],$$

where L_t = length at age 't';
L_∞ = asymptotic or maximum length;
k = the growth coefficient; and
t_0 = age or time when length theoretically equals zero.

Growth parameters were estimated for the sexes separately and combined. A likelihood ratio test was used to determine whether differences between female and male growth parameters were significant or if a single set of growth parameters described the data better (Kimura, 1980; Quinn and Deriso, 1999; Haddon, 2001) (SAS, vers. 8.0, SAS, Cary, NC).

Back-calculation is a method for describing the growth history of each individual sampled, and numerous variations in methods exist (see Francis, 1990, for a thorough review and Goldman, 2004, for a description and application to elasmobranchs). Back-calculations estimate lengths-at-previous-ages for each individual and should be used if sample sizes are small and if samples have not been obtained from each month (Goldman, 2004; Cailliet and Goldman, 2004). Cailliet and Goldman (2004) stated "the proportional relationship between animal length or disk width and the radius of the vertebral centrum among different length animals within a population is used as a basis for empirical relationships regarding population and individual growth, as is the distance from the focus to each annulus within a given centrum." Hence, choosing the appropriate method (based on the relationship between animal length or disk width and the radius of the vertebral centrum) is critical if back-calculated data are to be used for obtaining accurate life history parameter estimates from growth function models. Because smaller size classes were not well represented in our female sample and because our male sample size was small, lengths at previous ages were back-calculated from centrum measurements for both sexes and fitted with the von Bertalanffy growth function.

The relationship between CR and PCL for ENP salmon sharks was used to determine the most appropriate method for back-calculating previous length-at-age. To our knowledge, however, no studies on elasmobranch fishes have examined multiple back-calculation methods for statistical or biological accuracy in relation to vertebral sample data. To that end, four different proportion methods were used and compared with our sample length-at-age data. We first applied the standard Dahl-Lea direct proportions method (Carlander, 1969):

$$L_i(L_c / CR_c)\times CR_i, \quad (1)$$

where L_i = length at ring 'i';
L_c = length at capture;
CR_c = centrum radius at capture; and
CR_i = centrum radius at ring 'i'.

Next, we applied two modified versions of the Dahl-Lea method that use parameter estimates from the specific linear and quadratic fits that described the PCL-CR relationship. The linear-modified Dahl-Lea method (Francis, 1990) is

$$L_i = L_c\times\left[(a+bCR_i)/(a+bCR_c)\right], \quad (2)$$

where 'a' and 'b' are the linear fitted parameter estimates. The quadratic-modified Dahl-Lea method (Francis, 1990) is

$$L_i = L_c\times\left[(a+bCR_i+cCR_i^2)/(a+bCR_c+cCR_c^2)\right], \quad (3)$$

where 'a,' 'b,' and 'c' are the quadratic-fitted parameter estimates.

Ricker (1992) applauded Francis's (1990) back-calculation review paper, but like Campana (1990) suggested that the point of origin of proportional back-calculations should be related to a biologically derived intercept (i.e., length at birth). We, therefore, also applied Campana's (1990) "size-at-birth-modified" Fraser-Lee equation:

$$L_i = L_c+\left[(CR_i-CR_c)\times(L_c-L_{Birth})/(CR_c-CR_{Birth})\right], \quad (4)$$

where L_{Birth} = length at birth; and
CR_{Birth} = centrum radius at birth. (Based on Tanaka, 1980, 62.5 cm PCL was used for L_{Birth}.)

Von Bertalanffy growth parameter estimates were obtained from all individual and mean back-calculated length-at-age, and from a combination of back-calculated lengths-at-age and our sample data. A likelihood ratio test was used for all scenarios to determine whether differences between female and male growth parameters were significant or if a single set of growth parameters described the data better (Kimura, 1980; Quinn and Deriso, 1999; Haddon, 2001) (SAS vers. 8.0, SAS, Cary, NC). Back-calculated length-at-age results from all four methods were examined to see which best reflected our vertebral sample data.

The reproductive tracts of 64 female and 14 male salmon sharks were examined visually to assess their reproductive status. Females ranged in size from 71 to 209 cm PCL, and males ranged from 63 to 187 cm PCL. Clasper lengths were obtained from 12 of the males (from 91 to 187 cm PCL). Gross analysis of reproductive tracts and maturity determinations were made according to the methods of Pratt (1988), Gilmore (1993), Pratt and Tanaka (1994), Hamlett (1999) and Hamlett and Koob (1999).

Median precaudal length-at-maturity (MPCL) was determined by first coding female (n=64) and male (n=14) maturity data into binary form, with 0=immature and 1=mature. The binary data were fitted with a logistic regression model ("GLM" in S-Plus, Professional Release 1, Mathsoft Inc., Seattle, WA). The median precaudal length-at-maturity was then estimated as

$MPCL = -a/b$ (a=intercept, b=slope) (Mollet et al., 2000; Neer and Cailliet, 2001; Conrath and Musick, 2002; Conrath, 2004). To estimate percent maturity by length for the ENP salmon shark population, the 'a' and 'b' estimates (from the "GLM" model) were substituted into the equation; *percent mature* = $100 \times [(e^{(a+b \times length)})/(1+e^{(a+b \times length)})]$, and plotted against *PCL*. A nonparametric bootstrap (n=1000 replications) was conducted with S-Plus and 95% confidence limits were obtained from the 2.5th and 97.5th empirical percentiles.

Sperm storage in the oviducal gland has been documented in several shark species (Pratt, 1993). Oviducal glands were taken from six mature females, ranging in size from 180 to 192 cm PCL, caught in PWS waters in late July and late August, to determine the presence or absence of sperm. Samples were initially fixed in 10% formalin. Samples were rinsed extensively to remove fixative and then stored in 70% ethanol. Cross-sections along the entire length of each gland were trimmed, dehydrated in a graded series of ethanol, cleared in a limonene-based solvent, infiltrated with paraffin, and embedded in paraffin blocks. Serial sections (5 um) were prepared by using a rotary microtome, mounted onto poly-L-lysine-coated slides, dried, deparaffinized, rehydrated, and stained by using standard Harris hemotoxylin and eosin in order to examine general cellular detail (Hinton, 1990). Sections were examined for the presence of sperm by using a compound microscope at magnifications ranging from 100× to 1000×.

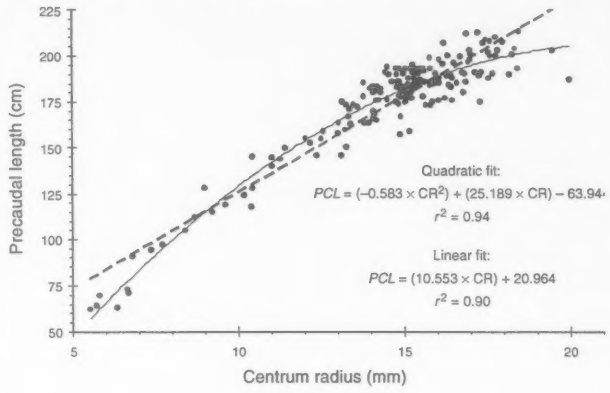

Figure 2

Relationship between centrum radius and precaudal length for eastern North Pacific salmon sharks (*Lamna ditropis*) showing significant fits given by linear and quadratic equations (sexes combined, n=182). PCL = precaudal length, CR = centrum radius.

Results

Length and weight equations

One hundred sixty-six of the 182 salmon sharks in this study were female, resulting in a F:M sex ratio of 10.4/1. Although females dominated our sample, the following equations for converting PCL into FL and TL appear to work equally well for both sexes.

$FL = 1.08 \times PCL + 6.91$ (r^2=0.99; n=138)

$TL = 1.15 \times PCL + 15.19$ (r^2=0.97; n=133).

Weight-length relationships for female and male salmon sharks we sampled in the ENP were $W = 8.2 \times 10^{-05} \times L^{2.759}$ (r^2=0.99, n=20) and $W = 3.2 \times 10^{-06} \times L^{3.383}$ (r^2=0.99, n=7) respectively, and $4.4 \times 10^{-05} \times L^{2.875}$ (r^2=0.99) for the sexes combined. A likelihood ratio test showed that separate equations for each sex describe the data better than a single equation for the sexes combined (χ^2=12.1; df=3; P<0.01).

Vertebral analysis

There was a slight curvilinear relationship between centrum radius (CR) and shark PCL (Fig. 2). A linear regression gave a significant fit to the data ($PCL = (10.553 \times CR) + 20.964$; r^2=0.90; P<0.0001); however, a quadratic equation produced a slightly better fit ($PCL = -63.944 + (25.189 \times CR) - (0.583 \times CR^2)$; r^2=0.94; P<0.0001) and a t-test showed the third parameter to be statistically significantly different from zero (t=10.32; df=181, P<0.0001). However, it was necessary to compare the mean back-calculated results from Equations 1 through 4 with the mean sample PCL data to see if the slightly better statistical fit of the quadratic equation translated into better biological accuracy for modeling growth.

Percent agreement (PA) among readers was 68.1% for the first set of blind reads and 72.0% for the second set, and the within-reader PA was 79.7% for reader one (the lead author) and 75.3% for reader two. Percent agreement (±one yr) was >95% for all reader comparisons. Agreement between and within reader age assessments was 100% until age 7 or 8, depending on the reader comparison. When grouped by 10-cm length increments, percent agreement was 100% for sharks ≤160 cm PCL, and 100% ±1 yr for sharks ≤180 cm PCL (Table 1). (PA and PA ±1 for length groups were virtually the same for both sets of between-reader comparisons, but slight differences occurred above 160 and 180 cm PCL, respectively.) The chi-square tests of symmetry gave no indication that differences between and within readers were systematic rather than due to random error (χ^2 test, P>0.05 in all cases).

Relative marginal increment (RMI) analysis showed that postnatal rings form annually between January and March. The smallest relative margins occurred in

Table 1

Percent agreement (PA) and PA ± one year, from the second set of "reads," for eastern North Pacific salmon sharks (*Lamna ditropis*) when placed into 10-cm precaudal length (PCL) groups.

PCL (cm)	Total no. read	No. agreed upon	No. agreed upon ±1	PA	PA ±1
60–70	5	5	5	100	100
70.1–80	2	2	2	100	100
80.1–90	0	—	—	—	—
90.1–100	4	4	4	100	100
100.1–110	1	1	1	100	100
110.1–120	5	5	5	100	100
120.1–130	3	3	3	100	100
130.1–140	1	1	1	100	100
140.1–150	6	6	6	100	100
150.1–160	9	9	9	100	100
160.1–170	11	8	11	72.7	100
170.1–180	30	22	30	73.3	100
180.1–190	55	38	53	69.1	96.4
190.1–200	34	22	32	64.7	94.1
200.1–210	12	4	10	33.3	83.3
>210	4	1	3	25.0	75.0
n	182	131	175	—	—
Percent agreement	—	—	—	72.0	96.2

February and March, followed by a consistent increase in RMI, and the largest relative margins occurred in December and January. There was no difference in this trend when immature and mature sharks were examined separately; therefore all ages classes were combined (Fig. 3).

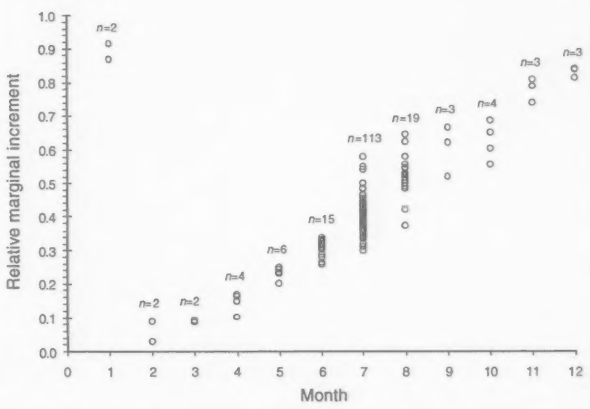

Figure 3

Results of relative marginal increment analysis showing that annulus formation occurs between January and March (n=176).

Vertebral age-at-length data from 166 female salmon sharks provided von Bertalanffy parameters of L_∞=207.4 cm *PCL*, k=0.17/yr, and t_0=−2.3 years (Fig. 4). When the von Bertalanffy growth function was fitted to the quadratic-modified Dahl-Lea back-calculations for females, the life history parameters were similar to those produced from the sample length data (Table 2). Vertebral age data from 16 males provided von Bertalanffy parameters of L_∞=182.8 cm PCL, k=0.23/yr, and t_0=−1.9 years (Fig. 4). Back-calculated lengths-at-age for male salmon sharks, either with or without sample data included, provided slightly lower k coefficients (=0.20), but similar L_∞ and t_0 parameters (Table 2). Vertebral age data for the sexes combined (n=182) provided von Bertalanffy parameters of L_∞=204.5 cm PCL, k=0.18/yr , and t_0=−2.2 years. A likelihood ratio test showed that separate von Bertalanffy growth models describe the data better for each sex than one model with the sexes combined (χ^2=29.1; df=3; P<0.001).

The quadratic-modified Dahl-Lea method (Eq. 3) represented most accurately the mean sample precaudal length-at-age data. It produced mean back-calculated lengths-at-age within 4.2 cm of mean sample lengths-at-age for female salmon sharks >120 cm PCL and within 8.8 cm for females <120 cm PCL (Fig. 5A). When applied to males, Equation 3 produced mean back-calculated lengths-at-age within 10 cm of mean sample lengths-at-age for sharks <120 cm PCL and >150 cm

Table 2

Von Bertalanffy growth parameters for female, male, and sexes combined for salmon sharks (*Lamna ditropis*) in the eastern and western North Pacific. Numbers in parentheses are standard errors. * = not independent.

	L_∞	k	t_0
Eastern North Pacific (present study)			
Females			
Vertebral sample data (n=166)	207.4 (2.5)	0.17 (0.01)	−2.3 (0.2)
Back-calculations (n=1814*)	205.3 (0.9)	0.18 (0.002)	−2.0 (0.03)
Mean back-calculation (n=21*)	210.0 (1.1)	0.17 (0.004)	−2.1 (0.09)
Back-calculations with sample data (n=1980*)	206.0 (0.8)	0.18 (0.002)	−2.0 (0.03)
Males			
Vertebral sample data (n=16)	182.8 (3.7)	0.23 (0.03)	−1.9 (0.3)
Back-calculations (n=161*)	183.9 (2.0)	0.20 (0.008)	−2.0 (0.1)
Back-calculations with sample data (n=177*)	184.2 (1.9)	0.20 (0.008)	−2.0 (0.1)
Combined			
Vertebral sample data (n=182)	204.5 (2.4)	0.18 (0.01)	−2.2 (0.2)
Western North Pacific (Tanaka, 1980)			
Females			
Vertebral sample data	203.8	0.14	−3.9
Males			
Vertebral sample data	180.0	0.17	−3.6

PCL. In between those lengths, however, the deviation from mean sample length-at-age increased to 16.5 cm (Fig. 5B). (The larger deviations for males were likely the result of the small sample size.) Lee's phenomenon did not occur in either sex with the quadratic-modified Dahl-Lea method.

Of the 64 female salmon shark reproductive tracts examined, 55 were fully mature, and 11 of the 14 males examined were mature. Mature female reproductive tracts consisted of a well-developed right ovary (the only functional ovary in lamniform sharks) containing various sizes of ova, large oviducts and oviducal glands, and expanded, heavily striated uteri with thickened walls. In stark contrast, the right ovary from immature female reproductive tracts was considerably smaller and had no signs of developing ova, the oviducal gland was virtually indistinguishable from the oviduct, and the uteri were extremely small, thin-walled, flaccid and completely smooth. Additionally, the epigonal organ in mature females was large and extended 2/3 the length of the uteri, whereas in immature females it was small and generally did not extend much past the anterior end of the uteri. No females examined were of an "intermediate"reproductive nature. Mature male reproductive tracts consisted of large well-developed testes, a thick epididymis leading to a coiled ductus deferens, rigid (fully calcified) claspers and well-developed siphon sacs. Immature male reproductive tracts consisted of small testes that were partially embedded in the epigonal organ, thin epididymis and straight ductus deferens, nonrigid claspers and poorly developed siphon sacs.

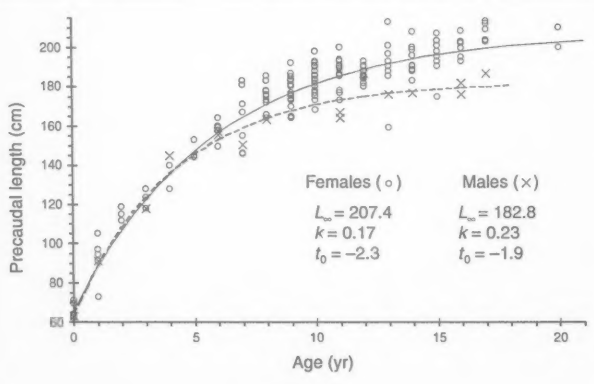

Figure 4

Von Bertalanffy growth curves fitted to female (n=166) and male (n=16) vertebral sample data for eastern North Pacific salmon sharks (*Lamna ditropis*). Estimates for parameters of the von Bertalanffy growth function are summarized.

Estimated median precaudal length-at-maturity (MPCL) for ENP female and male salmon sharks were 164.7 cm PCL and 124.0 cm PCL, respectively (Fig. 6). The smallest mature female observed was 164.0 cm PCL, and the largest immature female observed was 176.5 cm PCL (however, all other immature females were ≤163 cm PCL). None of

Table 3

Mean ages and precaudal lengths (PCL) for eastern North Pacific salmon sharks (*Lamna ditropis*) by location. CA = California; seAK = southeast Alaska.

	Females			Males		
	CA to seAK	Alaska	Combined	CA to seAK	Alaska	Combined
Age (yr)						
Mean	2	11	10	3	12	9
Range	0–5	5–20	0–20	0–7	6–17	0–17
PCL (cm)						
Mean	103.6	184.7	175.0	113.5	171.9	153.7
Range	62.2–153	144.8–213.4	62.2–213.4	63–150.5	155.4–187	63–187
n	20	146	166	5	11	16

the oviducal glands sampled from mature females contained spermatozoa. The smallest mature male observed was 155.4 cm PCL, and the largest immature male was 91.0 cm PCL. Based on age estimates from vertebral sample data, ages at MPCL range from 6 to 9 yr for females and from 3 to 5 yr for males. Ninety-five percent confidence limits (for percent mature) were very narrow for females <155 cm PCL and >172.5 cm PCL and for males <107.5 cm PCL and >132.5 cm PCL. The wide vertical confidence intervals around the calculated estimates of MPCL (Fig. 6) were caused by not having many observations for lengths around the estimated MPCL in the sample.

Age-length composition in the Eastern North Pacific

The mean age and length composition of ENP salmon sharks was latitude dependent (Table 3). Sharks between 0 and 7 yrs old, ranging from 62.2 to 153 cm PCL, were collected from southern California to southeast Alaska, with smaller individuals found in the southern part of that range. Sharks between 5 and 20 yrs old, ranging from 144.8 to 213.4 cm PCL, were collected from southeast Alaska up into the Gulf of Alaska, Prince William Sound, and the Bering Sea.

Discussion

Vertebral growth significantly increased with somatic growth (Fig. 2), which, along with the reliability of the RMI analysis

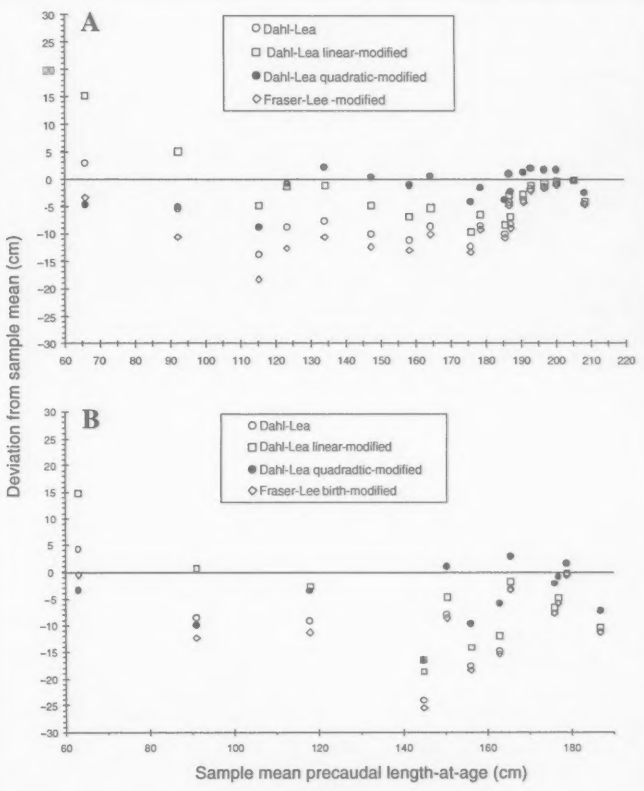

Figure 5

Mean deviation, from mean sampled precaudal length, of four proportional back-calculation methods for (**A**) female, and for (**B**) male eastern North Pacific salmon sharks (*Lamna ditropis*). Data points represent mean back-calculated lengths-at-age. An *x*-axis point on the zero line extending from the y-axis would represent zero deviation from the sample mean length-at-age.

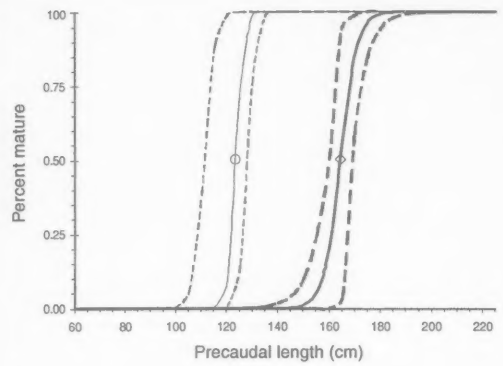

Figure 6

Percent versus precaudal length curves for eastern North Pacific female (heavy lines) and male (light lines) salmon sharks (*Lamna ditropis*) with 95% confidence bands on percent mature. Diamond and circle show estimates of median precaudal length at maturity (MPCL) for females and males respectively. Confidence bands (dotted lines) are 2.5th and 97.5th empirical percentiles obtained by bootstrapping.

(Fig. 3), demonstrates that vertebral growth patterns are a reliable indicator of age in salmon sharks. Precision was high between and within readers with limited differences (Table 1) that were attributable to random error. These results provided us with a high degree of confidence in the age assessments determined from the von Bertalanffy growth model (with vertebral sample data), and hence in the resulting life history parameter estimates.

Tanaka (1980) and Nagasawa (1998) stated that salmon sharks produce one ring per year, but a RMI analysis was not provided in their studies. Our RMI analysis verified an annual periodicity of banding patterns for salmon sharks, ranging from 73 cm to 213.4 cm PCL and encompassing ages 1 to 20 for females and ages 1 to 17 for males. The major period of growth occurs from May through November, slowing some as January approaches (Fig. 3). A brief cessation, or extreme slowing, of growth (ring formation) occurs between January and March, and growth increases again in April–May. Although we were able to examine specimens from every month of the year, additional samples from December through April would enhance RMI precision.

The similar von Bertalanffy growth parameter estimates generated from female vertebral sample data, back-calculated data, and the combination of the two indicated that sample size was sufficiently large and encompassed the known size range of the species. Von Bertalanffy parameters for males would have been improved with a larger sample size that would have reduced the difference between values of 'k' from vertebral sample and back-calculated data (Table 2). More samples would likely have had little influence on L_∞ for either sex because salmon sharks close to maximum length were examined in the present study. It is possible that the discrepancy between female and male sample sizes could have influenced the outcome of the likelihood ratio test. However, with the differences in observed maximum length (and estimated L_∞) between the sexes and the small standard errors associated with the male von Bertalanffy estimates (Table 2), it is unlikely that an increased male sample size would have altered the test result.

The error associated with back-calculated length *versus* the actual length at a given age has been a focal point of papers by Campana (1990), Francis (1990) and Ricker (1992) and prompted our evaluation of several proportional back-calculation methods. There was a statistically valid reason for using the quadratic-modified Dahl-Lea (Eq. 3) over the linear-modified Dahl-Lea (Eq. 2) (see "Results" section); however, the only way to cross-compare all four back-calculation methods in a biologically meaningful way was to apply all of them to the vertebral sample data. Both modified Dahl-Lea equations were more accurate in representing the mean sample length-at-age data than the standard Dahl-Lea or the size-at-birth-modified Fraser-Lee equations (Fig. 5, A and B). However, the quadratic-modified Dahl-Lea was the best predictor of prior length-at-age (i.e., best resembled our vertebral sample length-at-age data). Although these back-calculation results are, of course, dependent on the assumption that growth has not significantly changed over time, and are applicable only to salmon sharks, they demonstrate the importance of choosing the appropriate method to minimize error, which results in a greater ability to accurately model growth. Accurate growth parameters will result in more accurate demographic parameters and stock assessments, leading to more responsible management. Even greater confidence could be achieved if animals collected in the past were available because they would allow a direct comparison of size-at-age then and now in order to verify that the growth rate for the salmon shark has not changed.

Our results show that salmon sharks in the ENP attain their maximum size at a faster rate (k) than those from the WNP (Table 2). We were unable to test the statistical significance of these differences because neither Tanaka (1980) nor Nagasawa (1998) provided point estimates and standard error values for their WNP data. There were, however, significant resultant biological differences present. Both female and male salmon sharks reach first age at sexual maturity approximately 2 years earlier in the ENP than in the WNP. Female salmon sharks in the WNP mature between 8 and 10 years of age (Tanaka, 1980; Nagasawa, 1998). From the reproductive tracts examined, we found that female salmon sharks in the ENP reach sexual maturity between ages 6 and 9. Although age at first maturity was earlier in the ENP, length at maturity appears to be similar; 160–180 cm PCL in the WNP, and a MPCL = 164.7 cm PCL in the ENP (Fig. 6). Male salmon sharks in the WNP mature at approximately

5 years of age and 140 cm PCL (Tanaka, 1980; Nagasawa, 1998). From our small sample size, the logistic method provided an estimate that male salmon sharks in the ENP mature between the ages of 3 and 5, and MPCL=124.0 cm PCL (Fig. 6). Clasper lengths did not enhance the ability to determine male length at sexual maturity because specimens around the calculated MPCL were not sampled. Although the MPCL for males indicates a smaller length at maturity, our confidence in the accuracy of the male MPCL estimate (due to sample size) is low (Fig. 6).

The lack of spermatozoa in any of the oviducal gland samples analyzed is not surprising considering no lamniform shark examined to date has shown evidence of sperm storage (Pratt, 1993). However, specimens we examined were specifically chosen from the time period of late July through late August when mating was likely to have been taking place (Goldman and Human, 2005; Goldman and Musick, in press). A larger sample size taken through that time period and extending through September may resolve the question better regarding the suspected residence time of sperm in the oviducal gland of lamniform sharks—a residence time that is probably only a few days timed with actual mating activity (Pratt, 1993). The large number of eggs produced to feed oophagous young (Wourms, 1977; Gilmore et al., 1983; Gilmore, 1993) could have "flushed" any stored sperm out of the oviducal gland (Pratt, 1993).

In the WNP, salmon sharks are born in the spring of the year and pups range in length from 60 to 70 cm PCL (Tanaka, 1980; JAMARC, 1980; Nagasawa, 1998). Litter size in the WNP is up to 5 pups, and the ratio of male to female is 2.2:1 (Tanaka, 1980). Our data support a similar timing for pupping and for length at birth in the ENP, but no pregnant females were taken during the course of our research and therefore information on litter size and pup sex ratio is not available for the ENP.

Nagasawa (1998) characterized salmon shark growth through age ten as follows; after birth, "they grow to between 90 and 105 cm PCL by the next spring ... subsequent annual growth is 10–15 cm per year up to age-4 fish." He stated, in addition, that females and males attain an average length of 173 cm PCL and 163 cm PCL, respectively, by age 10. Our data indicated that the average growth rate (cm per year) for salmon sharks in the ENP is very similar to the rate in the WNP through age 4. By age 5, ENP females are growing at a considerably faster rate than WNP females, reaching an average of 185.4 cm PCL by age 10 (Table 4). Male salmon sharks in the ENP appear to begin outgrowing their WNP counterparts by age 4 (Table 4). Salmon shark longevity appears to be similar in the ENP and WNP. Maximum observed age for females in our study was 20, whereas it was 17 in the WNP (Tanaka, 1980); however, maximum observed age for males was only 17, whereas it was 25 for the WNP. We were unable to obtain samples of male salmon sharks at or near maximum age because of our small sample size.

Salmon sharks in the ENP and WNP attain the same maximum length (about 215 cm PCL for females and about 190 cm PCL for males). However, WNP salmon sharks take longer to reach a given length than those in the ENP (Table 4). Additionally, the weights for the same length females and males are considerably different except at male maximum length, which appears to be equivalent to females of similar length. Our data indicate that the weight-at-length differences between ENP and WNP salmon sharks begin at approximately 110 cm PCL for females, and at 140 cm PCL for males (Fig. 7, A and B). Although a greater sample size could enhanced precision and may alter the equations presented in the present study to some degree, the data show that weight-at-length is particularly greater for adult salmon sharks in the ENP than in the WNP. As with the von Bertalanffy growth models, it is possible that the discrepancy between female and male sample sizes may have influenced the outcome of the likelihood ratio test. However, the sample-size discrepancy was not as large as with the growth curve data and standard errors were small for both sexes; therefore it is unlikely that an increased male sample size would have altered the test result.

Although season and size at birth appear to be similar for salmon sharks in the ENP and WNP, the locations where females pup are different. A salmon shark pupping and nursery ground exists along the transitional boundary of the subarctic and central Pacific currents (Nakano and Nagasawa, 1996). Our data indicate that a second pupping and nursery ground exists, ranging from southeast Alaska to the northern end of Baja California, Mexico (central California being the most common area for ages zero and one [see Table 3]). Ages zero through 5 were caught only between southern California and southeast Alaska, and ages 5 and above were caught only in Alaska waters. The latitudinal size segregation observed in the ENP indicates that pregnant females may move south along the California coast to give birth in the spring.

The high degree of sexual segregation across the Pacific Basin (Nagasawa, 1998; Goldman and Musick, in press), along with the age- and length-dependent latitudinal distribution are important factors in pursuing responsible management and conservation of the salmon shark. Although latitudinal migrations and movements are documented in this species (Iino, 1939; Kosugi and Tsuchisaki, 1950; Tanaka, 1980; Balgaderov, 1994; Nakano and Nagasawa, 1994; Weng et al., 2005), they are still poorly understood. Similarly, although trans-Pacific movements have been inferred from fisheries bycatch data (Goldman and Musick, in press), there has yet to be documentation of individuals moving across the North Pacific. However, the degree of the sexual segregation by itself (in regard to finding a mate) would seem to indicate that movements across the Pacific are likely.

Another critical element for successful management of salmon sharks is stock structure, which is not well understood at this time; however a study of population

Table 4

Mean precaudal length (PCL) at age for salmon sharks (*Lamna ditropis*) in the eastern and western North Pacific (ENP and WNP). Ranges (or individual lengths for some males) are given for sharks from the present study.

	Females			Males	
Age	WNP* mean PCL	ENP mean PCL	ENP range	WNP* mean	ENP individual or range
0	62.5	65.9	62.2–71.1	62.5	63.0
1	97.5	92.1	73.0–105.0	97.5	91.0
2	110.0	115.3	112.0–119.0	110.0	—
3	122.5	123.3	118.0–128.0	122.5	118.0
4	135.0	134.0	128.0–140.0	135.0	145.0
5	141.3	147.3	144.0–153.0	139.7	—
6	147.7	158.1	149.7–164.0	144.3	155.4–157.0
7	154.0	164.1	145.9–183.0	149.0	150.5
8	160.3	175.7	164.0–185.4	153.7	163.0
9	166.7	178.4	164.0–192.0	158.3	—
10	173.0	185.4	168.0–198.0	163.0	—
11	177.4	187.0	173.0–200.0	164.2	164.0–176.0
12	181.8	186.7	180.0–193.0	165.3	187.0
13	186.2	190.8	159.9–213.0	166.5	176.0
14	190.6	192.9	183.0–208.0	167.6	177.0
15	195.0	196.7	175.0–207.1	168.8	—
16	199.4	200.1	193.0–208.3	169.9	176.0–182.0
17	203.8	208.3	203.0–213.4	171.1	187.0
18	—	—	—	172.2	—
19	—	—	—	173.4	—
20	—	205.1	200.0–210.2	174.5	—
21	—	—	—	175.7	—
22	—	—	—	176.8	—
23	—	—	—	178.0	—
24	—	—	—	179.1	—
25	—	—	—	180.3	—

* Mean length-at-age for WNP calculated from information in Nagasawa (1998), L_∞ was used as mean length at age 17 for females and as mean length at 25 for males.

genetics is currently underway. Current information from the western and central North Pacific implies that salmon sharks constitute a single stock, but there is no current information for the Japan Sea or the eastern North Pacific (Sano, 1962; Tanaka, 1980; Blagoderov, 1994; Nagasawa, 1998). New technologies such as archival and pop-up satellite transmitters should provide documentation of movements and migrations (Weng et al., 2005) and key information as to whether regional or international conservation and management plans are needed.

Stock structure may be an important factor in the differential growth rates between ENP and WNP salmon sharks. However, ecological differences between the ENP and WNP could also be responsible for the observed differences. Young salmon sharks appear to move from temperate waters of the U.S. west coast into the Alaska Gyre and the Gulf of Alaska as they approach adulthood, which is when their growth rate begins to exceed that of their WNP counterparts. These waters are extremely productive (Strub et al. 2001), and abundant food resources may be the key factor in the differences in growth rate, age-at-maturity, and weight-at-length observed between ENP and WNP salmon sharks.

A high degree of variability exists in the periodicity of ring and growth band formation within and among taxonomic groups of elasmobranchs, and much of the variation observed in several lamniform sharks has not yet been explained (Branstetter, 1990; Branstetter and Musick, 1994; Wintner and Cliff, 1999). For example, Cailliet et al. (1983) stated that shortfin mako sharks (*Isurus oxyrinchus*) from the Pacific appear to form growth rings annually, whereas Pratt and Casey (1983) stated that Atlantic specimens appeared to produce two

rings per year. However, validation of single annual ring (band pair) deposition has now been demonstrated from recaptures of oxytetracycline(OTC)-injected mako sharks (Natanson et al., in press), as well as with a new technique where bomb radiocarbon is used to validate ages in long-lived sharks (Campana et al., 2002; Ardizzone et al., in press).

Lamnid sharks are endothermic (Carey et al., 1985; Lowe and Goldman, 2001), and prior to the Campana et al. (2002) and Ardizzone (in press) radiocarbon studies, it appeared that lamnid sharks might possess fairly high growth rates (growth coefficient, k) compared to other sharks, particularly those that grow to a large size (Musick, 1999). However, with mako sharks now appearing to have a growth coefficient around 0.072/yr (sexes combined)(Cailliet et al. 1983), the validation of porbeagle shark (*Lamna nasus*) age and growth (k=0.06 and 0.08/yr for females and males, respectively) (Natanson et al. 2002), and estimates of white shark (*Carcharodon carcharias*) growth coefficients ranging from 0.058 to 0.071/yr (for sexes combined)(Cailliet et al., 1985; Wintner and Cliff, 1999; Malcolm et al. 2001), salmon sharks appear to be the species that possess the higher growth rates (Tanaka, 1980; present study). Salmon sharks are not only endothermic, but also appear to be homeothermic (i.e., defend a specific body core temperature regardless of ambient temperature) (Goldman et al., 2004). This uncommon physiological trait for a shark, combined with a diet that includes many lipid-rich species (e.g., salmonids), may influence the growth rate of this species, but endothermy does not appear to be correlated with faster growth rates in lamnid sharks (Goldman, 2002).

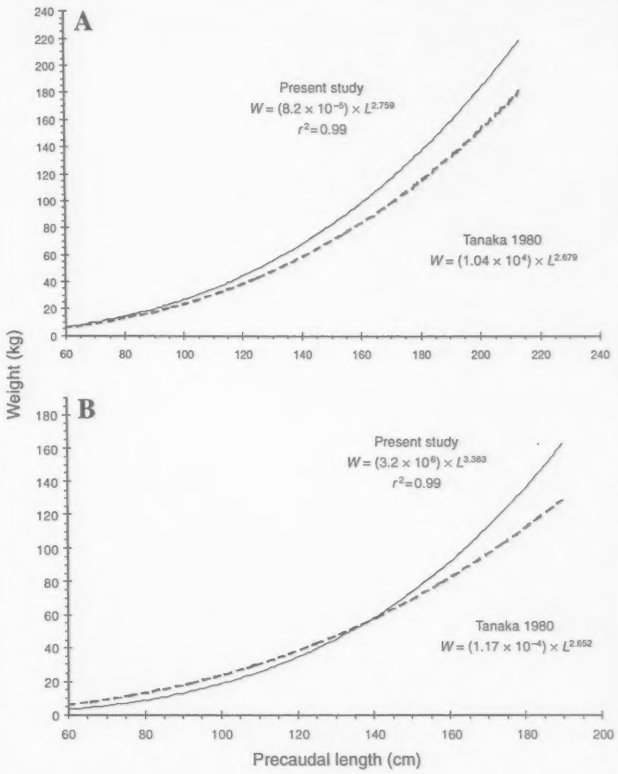

Figure 7

Comparison of weight-length relationships of eastern and western North Pacific salmon sharks (*Lamna ditropis*) (**A**) females, and (**B**) males. Western North Pacific data were obtained from Tanaka (1980) and Nagasawa (1998).

The most recent demographic analysis has indicated that salmon shark populations in the eastern and western North Pacific are stable at this time (Goldman, 2002) and current research is beginning to assess standing stock and population numbers, as well as examine the stock structure of salmon sharks in the eastern and western North Pacific in order to further responsible management of the species. The differences in growth rates and strong sexual segregation across the North Pacific basin and an unknown rate of current bycatch complicate management of this species.

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Potential causes of mortality for horseshoe crabs (*Limulus polyphemus*) during the biomedical bleeding process

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Biomedical companies catch and bleed horseshoe crabs for the production of *Limulus* amebocyte lysate (LAL), a product used for protecting public health (Berkson and Shuster, 1999). LAL is a clotting agent, derived solely from horseshoe crab blood cells, which is used to detect the presence of pathogenic gram-negative bacteria in injectable drugs and implantable medical and dental devices (Mikkelsen, 1988; Novitsky, 1991). In addition, LAL is used in many diagnostic tests for such illnesses as gram-negative bacterial meningitis and typhoid fever (Ding and Ho, 2001). Because the LAL test allows one to detect femtogram levels of endotoxin (Ding and Ho, 2001), it is the most effective test for detecting endotoxin contamination, and its increasing use in medical and pharmaceutical laboratories makes it a highly valued product.

The biomedical industry harvested approximately 260,000 horseshoe crabs in 1997 (HCTC[1]) for the production of LAL. By 2000, the number of horseshoe crabs bled had increased less than 1% (ASMFC[2]). However, approximately 25% of the horseshoe crabs landed for biomedical purposes were rejected for use, and about 45% of those rejected were rejected because they were injured (ASMFC[2]). Some mortality is likely related to harvest injuries, but this rate of mortality is unknown. At current harvest levels, mortalities from biomedical collection and bleeding methods may not be negligible. Mortalities inflicted by the biomedical industry on the horseshoe crab population come in addition to mortalities caused by the commercial fishery, where horseshoe crabs are harvested for use as bait for eel (*Anguilla rostrata*) and whelk (*Busycon* spp.) fisheries (Walls et al., 2002). Furthermore, numerous migratory shorebird species feed on horseshoe crab eggs (Clark, 1996) and adults (Botton and Loveland, 1989, 1993) to fuel their migration from South American wintering grounds to their Arctic breeding grounds (Clark, 1996). Concern has arisen over horseshoe crabs, in part because of local population declines, increased catch and effort, and the need for a superabundance of horseshoe crab eggs for shorebirds, all of which necessitate a conservative, risk-averse management approach (ASMFC[2]). It is important to not only investigate the effects of the commercial fishery harvest and natural predation on horseshoe crab stocks but to comprehensively assess the impact of the biomedical industry take on horseshoe crab population viability. Reducing mortalities from the biomedical industry may aid in conserving the horseshoe crab population, as well as in reducing the magnitude of conflict between this industry, the commercial fishery, and environmentalists. To achieve this goal, further investigation into the biomedical bleeding process and its effects is required.

Throughout the typical biomedical bleeding process, horseshoe crabs are subjected to a variety of potential stressors (i.e., air exposure, increased temperature, handling, blood loss, trauma, etc.). Each LAL producer has its own bleeding process involving different methods of capture, distance and method of travel to the bleeding facility, different holding times and conditions, different bleeding methods, and methods of returning bled crabs that are most appropriate to that company's location and facility set-up (Walls and Berkson, 2003). An example of one version of the biomedical bleeding process begins with the collection of the horseshoe crabs by trawling, dredge, or hand-harvest methods (HCTC[1]). Animals may be held on the deck of a boat or in containers for several hours during collection, transported to the bleeding facility in trucks (that may or may not be air-conditioned), held in the

[1] HCTC (Horseshoe Crab Technical Committee). 1998. Status of the horseshoe crab (*Limulus polyphemus*) population off the Atlantic Coast, 9 p + figures and tables. Horseshoe Crab Technical Committee, Atlantic States Marine Fisheries Commission, 1444 Eye Street, NW, Sixth Floor, Washington, DC 20005.

[2] ASMFC (Atlantic States Marine Fisheries Commission). 2002. Review of the fishery management plan for horseshoe crab (*Limulus polyphemus*). Horseshoe Crab Plan Review Team, Atlantic States Marine Fisheries Commission, 1444 Eye Street, NW, Sixth Floor, Washington, DC 20005.

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cold room of a laboratory for several hours at an air temperature of 16–18°C, bled for a period of time, and then held in the coldroom or in a truck until transport back to the dock (Grogan[3]; Walls[4]). Horseshoe crabs that are returned to the ocean are transferred onto a boat and returned to their approximate point of capture within 72 hours of their collection (HCTC[1]). The numerous stressors to which the horseshoe crabs are exposed throughout the biomedical bleeding process likely have an effect on their mortality rates, both for horseshoe crabs used for bleeding and for those crabs collected, but rejected for use.

Additional sources of stress also may arise from the bleeding methods used by some biomedical companies. The bleeding protocols at some facilities involve gravitationally withdrawing blood from the heart (punctured with a large gauge needle) until blood flow slows to an intermittent drip (Grogan[3]; Walls[4]). One study reported a range of extracted blood to be from 8.4 mL to 218.7 mL in male horseshoe crabs (Walls, 2001) and up to 267.8 mL in females (Walls[5]). Unfortunately, biomedical companies do not know how much blood horseshoe crabs of a given size possess and how much can be safely extracted. Thus, extracting the reported upper ranges of blood volume from males and females may cause stress or mortality.

Several studies have estimated mortality associated with the biomedical bleeding process to be between 8% and 20% (Rudloe, 1983; Thompson, 1998; Kurz and James-Pirri, 2002; Walls and Berkson, 2003). These studies either simulated the biomedical bleeding process (Rudloe, 1983; Kurz and James-Pirri, 2002) or monitored mortality of a given number of horseshoe crabs bled by a biomedical company (Thompson, 1998; Walls and Berkson, 2003). Although the results provide information on mortality rates associated with a biomedical companies' procedures, they provide no guidance on how biomedical companies may reduce mortality rates.

Rather than quantifying mortality rates associated with the biomedical bleeding process as a whole, we isolated and tested certain elements of the bleeding process to gain information on the potential causes of mortality for horseshoe crabs used in the biomedical industry. The objectives of this study were 1) to quantify mortality associated with blood extraction at set levels and 2) to quantify mortality associated with stress from simulated transport and holding procedures, combined with the stress of blood extraction at the same levels as in the first objective.

[3] Grogan, W. 2003. Personal commun. Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State Univ., Blacksburg, VA 24061.

[4] Walls, B. 2001. Personal commun. Center for Environmental Studies, Virginia Commonwealth Univ., 1000 West Cary Street, Box 843050, Richmond, VA 23284.

[5] Walls, B. 2001. Unpubl. data. Center for Environmental Studies, Virginia Commonwealth Univ., 1000 West Cary Street, Box 843050, Richmond, VA 23284.

Methods

Adult horseshoe crabs were obtained from Cambrex BioScience Walkersville, Inc., a commercial biomedical company on 9 July and 28 August 2003. Horseshoe crabs were captured by using a standard trawling procedure off the coast of Ocean City, Maryland (Hata and Berkson, 2003). After capture, horseshoe crabs were transported in an air-conditioned van to the Horseshoe Crab Research Center (HCRC) at Virginia Polytechnic Institute and State University in Blacksburg, Virginia. The animals were maintained in a recirculating aquaculture system in appropriate environmental conditions (Brown and Clapper, 1981) and monitored daily, but not fed. The animals were acclimated to the aquaculture conditions for one week, during which time they were tagged, sexed, and measured. Tagging involved drilling two 3/32″ holes into the prosoma at its thinnest point and attaching a laminated oval fish tag (Floy Tag, Seattle, WA) with two 3/32″ wide cable ties. Measurement consisted of recording the inter-ocular width (IO), the distance between the inside margins of the horseshoe crab's compound eyes, and the prosomal width (P), which is the distance across the horseshoe crab's carapace. Horseshoe crabs used for both bleeding mortality studies had a mean IO width of 14.0 cm and ranged from 10.5 to 19.0 cm; as well as, an average P width of 23.5 cm that ranged from 17.5 to 35.0 cm. Only animals that were uninjured (i.e., no cracked carapaces or missing legs, etc.) and free of epibionts were used in the two mortality experiments.

Experiment 1

Horseshoe crabs from the July collection were used in experiment 1. From this collection, 100 males and 100 females were selected to test only the effects of various levels of blood extraction on mortality. This sample of horseshoe crabs was termed the "lower-stressed" group because they were not exposed to external stressors associated with simulated holding and transport. The selected horseshoe crabs ranged in size to provide a representative sample of animals bled by some biomedical companies (Grogan[3]; Walls[4]). Horseshoe crabs were arbitrarily assigned to one of five bleeding treatments: 1) unbled crabs (control), 2) crabs bled 10% of their predicted total blood volume, 3) crabs bled 20% of total blood volume, 4) crabs bled 30% of total blood volume, and 5) crabs bled 40% of their predicted total blood volume. Each bleeding subgroup comprised equal numbers of males and females. Predicted blood volume was calculated by using the relationship between blood volume and IO width (Hurton et al., 2005):

$$H = 25.7e^{0.1928\,(IO)},$$

where H = hemolymph volume in mL; and
IO = interocular width in cm.

The bleeding process of experiment 1 involved removing horseshoe crabs from their holding tank, positioning

them in a bleeding rack, and disinfecting the surface of their arthrodial membrane with a 70% ethanol-soaked cotton swab. An 18-gauge needle was inserted through the arthrodial membrane into the cardiac sinus to extract the predetermined amount of blood. The animals were then placed in a holding container without water for 15–20 hours. Time out of water approximated the duration that horseshoe crabs may be out of water after bleeding at some biomedical bleeding facilities (Grogan[3]; Walls[4]). Bled horseshoe crabs were also not immediately put back in water to prevent them from absorbing water and regaining their blood volume. Temperatures to which horseshoe crabs were exposed were recorded using a temperature logger. Air temperature during this time was stable at 21°C. Subsequently, the animals were returned to their holding tanks and mortality was monitored for two weeks.

Experiment 2

Horseshoe crabs from the August collection were used for experiment 2. Because of difficulty in obtaining the desired 200 study animals, slightly fewer (195) horseshoe crabs were selected for this experiment. This sample of 110 males and 85 females was used to test the effects of various levels of blood extraction on mortality in the presence of external stressors. These animals represented the "higher-stressed" group because they were exposed to additional stressors to which the first group was not. The external stressors (i.e., air exposure, increased temperature, etc.) were those derived from simulated transport and holding procedures during a simulated biomedical bleeding process. Because there is great variability in transport and holding procedures and conditions, we simulated a protocol that would demonstrate horseshoe crab response under relatively poor conditions.

During the week of acclimation to the aquaculture system, the horseshoe crabs were held in the same conditions and handled in the same manner as group 1. Horseshoe crabs were assigned to one of five bleeding treatments used in experiment 1. The bleeding process of experiment 2 involved removing horseshoe crabs from their holding tank and placing them in holding containers located outside where they were exposed to air, sun, and increased temperatures. During this 6-hour period, the air temperature rose from 21°C to 29°C to simulate time on the deck of a trawler. Next, the animals were moved into a small moving truck, which was not air-conditioned. This phase simulated transportation to the bleeding facility and holding time until placement into the coldroom of the laboratory. During this 4-hour period, the outdoor temperature increased to 31°C and the temperature inside the closed truck peaked at 36°C. The animals were then transferred to the HCRC's tank room where they remained for 16 hours at 21°C. This phase mimicked the holding time in a coldroom prior to bleeding. The animals were bled according to their assigned treatment and in the same manner as in experiment 1. During this 8-hour period, the room temperature was 22°C. Once the bleeding was completed, the horseshoe crabs were moved back into the truck for 13 hours to simulate holding time and transport to the boat. The overnight temperature inside the truck dropped from 24°C to 20°C. In the morning, the horseshoe crabs were returned to the water in their recirculating aquaculture tanks and mortality was monitored for two weeks.

Statistical analyses

Fisher's exact test was used to evaluate 1) differences in mortality of unbled horseshoe crabs between the higher-stressed and lower-stressed groups, and 2) differences in mortality of bled crabs between the lower-stressed and higher-stressed groups. Logistic regression was used to examine the association between mortality and bleeding in the higher-stressed group. Data were also assessed to see if an increase in the volume bled was correlated with an increase in mortality by pooling the frequency of mortalities between males and females in each bleeding treatment and testing these values against bleeding percentage by employing a regression with a fitted quadratic curve.

Results

Experiment 1

No horseshoe crab mortality occurred in any of the five treatments in the lower-stressed group, indicating that post-bleeding mortality in *Limulus* did not arise solely from the effects of blood loss. Hence, our initial hypothesis that substantial blood loss was the principle cause of mortality in horseshoe crabs was rejected.

Experiment 2

There were a total of 14 mortalities distributed throughout the bleeding treatments among horseshoe crabs under higher stress conditions (Table 1). All mortalities occurred within the first seven days of the study. Bled horseshoe crabs had an overall mortality rate of 8.3% compared to the 2.6% mortality rate of unbled crabs, suggesting a relationship between mortality and bleeding under higher-stressed conditions (n=195; P=0.0088) (Table 1). The bleeding variable was significant in the logistic regression model (P=0.0160); yet, sex was not a significant variable (n=156; P=0.6100); seven deaths in total occurred in each sex category (Table 1). Mortality rates reached 13.6% for males bled 30% of blood volume and female mortality was as high as 29.4% in the subgroup bled 40% of blood volume (Table 1). With male and female mortalities pooled, the frequency of mortality increased as the percentage of blood taken from the crabs was increased (n=5; P=0.006; r^2=0.994) (Table 1).

Comparison of the two experiments

With no mortalities in the unbled treatment of the lower-stressed group and one mortality in the unbled treat-

Table 1

Comparison of mortalities observed in the higher-stressed group between unbled horseshoe crabs and horseshoe crabs bled at different levels of total blood volume (n=195).

	Mortality (no.)			Mortality (%)		
% bled	Male	Female	Total	Male	Female	Total
0%	1	0	1	4.5	0	2.6
10%	0	1	1	0	5.9	2.6
20%	2	0	2	9.1	0	5.1
30%	3	1	4	13.6	5.9	10.3
40%	1	5	6	4.5	29.4	15.4
Total	7	7	14	6.4	8.2	7.2

ment of the higher-stressed group, mortality rates were not significantly different between unbled crabs in the two bleeding experiments (n=79; P=0.4937). Of the bled crabs, there was a mortality rate of 0% in the lower-stressed group and a rate of 8.3% in the higher-stressed group, indicating a significant difference in mortality rates between the two groups (n=316; P<0.0001).

Discussion

According to our study of 395 horseshoe crabs, no impact on mortality was observed with blood extraction up to 40% of the horseshoe crab's blood volume under conditions of lower stressors. Any mortality associated with this maximum level of bleeding (40% of total blood volume) was so low that it was not captured by our sample size of 40 individuals per bleeding treatment. This result also may indicate that despite hypovolemia, or low blood volume, and altered blood chemistry (Hurton, 2003), horseshoe crabs may be relatively tolerant of the removal of a large amount of blood in the absence of other stressors.

Horseshoe crab mortality, however, was significant in the presence of higher levels of stress, including external stressors (i.e., lengthy air exposure, elevated temperatures, etc.) as applied in our study. Novitsky's (1991) aquarium studies reported that up to 30% of blood volume can be safely extracted, indicating that the animals in that study may have been similar to our lower-stress group; however, details regarding harvest method, transport, and handling procedures were not specified (Novitsky, 1991). It is likely that, under different conditions, horseshoe crab response to blood extraction may be altered. Findings from our study indicated that a combination of external effects and sublethal hemorrhaging may result in significantly increased mortalities, possibly from a synergistic interaction between the two types of stressors. Studies in other species exposed to various stressors have also indicated that mortality may be affected by a synergistic combination of effects from multiple stressors (Schisler et al., 2000; Schulz and Dabrowski, 2001; Hatch and Blaustein, 2003). Such results emphasize the importance of considering the cumulative impact of multiple stressors.

Although the data in our study indicated a significantly increased mortality rate in the higher-stressed group as opposed to the lower-stressed group, it is important to note the variability in mortality throughout the treatments. It was observed that the male and female horseshoe crabs had different and variable responses in the different bleeding treatments. For example, males had two and three mortalities in the 20% and 30% bleeding treatments, respectively; yet, only one mortality in the 40% treatment (Table 1). Females appeared to have a spike in mortality in the 40% treatment (Table 1). When viewing the data this way, the variability in mortality could be in part due to the health and precollection stress level of a horseshoe crab, which can differ from its counterparts. Different conditions may lead to different responses and not every individual will react exactly alike. More than likely, much of this variability is an artifact of a small sample size. Once the male and female data were pooled, total mortality appeared to proceed in an increasing trend as bleeding amount increased (Table 1). If sample size were increased enough, perhaps a strong trend would emerge in mortality between males and females, or perhaps not. Further investigation with larger sample sizes and a different experimental design could shed light on a possible trend in male and female mortality rates and whether one sex may be more susceptible to mortality than the other.

The combination of multiple stressors could also affect not only mortality, but possibly the amount of blood able to be extracted by the biomedical industry. It is possible that horseshoe crabs exposed to higher levels of external stress, such as greater lengths of time to air and higher temperatures, would lose moisture from their exposed gills and possibly become dehydrated and thus have a decreased blood volume such that a smaller amount of blood is available for extraction. It is unknown how this would affect the harvest of horseshoe crab blood for the production of LAL. Dehydration rates in horseshoe crabs are unknown, but have been examined in a freshwater crayfish (*Austropotamobius pallipes*) which is a facultative air breather that can survive about three days out of water (Taylor et al., 1987). Taylor et al. reported that when exposed to air (70–80% relative humidity) for 27 hours, crayfish became dehydrated and had a 25% decreased blood volume. When exposed to water-saturated air (100% relative humidity), crayfish did not have a decrease in blood volume. Similarly, horseshoe crabs exposed to water-saturated air may be less likely to become dehydrated, thereby possibly decreasing the effects of one type of stress.

The results presented in the present study provide insight into the possible combined effects of blood extraction and external stressors associated with biomedical

transport and holding methods on horseshoe crab mortality. Of the various protocols followed in the bleeding process, we simulated an example of transport, holding, and bleeding methods that provided poor conditions for the horseshoe crabs and that resulted in significant mortality rates. In the typical biomedical bleeding process, horseshoe crabs undergo time on a boat deck or in collection bins, transport, storage in a coldroom, bleeding, holding time, and transport back to the ocean. At each of these stages of processing, the animals are exposed to a number of stressors of varying magnitudes, including exposure to air for extended time periods, elevated temperatures, dehydration, hypovolemia, and likely other unknown stressors. However, these factors can be controlled to a certain extent by providing conditions for the horseshoe crabs that could alleviate much of the stress. For example, holding horseshoe crabs in an air-conditioned environment (i.e., during time in the truck and in the cold room), covering the animals with wet burlap while outside to keep them moist and shaded from the sun, and increasing the relative humidity of the air in the holding room could help to decrease the physiological stress the animals experience throughout the bleeding process. Because blood volume estimates have recently been determined (Hurton et al., 2005), the blood volume extracted by biomedical companies potentially could be optimized according to average stress conditions and attempts could be made to decrease stress as much as feasibly possible, including keeping horseshoe crabs hydrated and bleeding a prescribed amount from individuals, as opposed to withdrawing a wide range of blood volumes. Another important consideration is stress arising from harvest methods. Our study did not test the effects of this factor, but it may have an effect on the condition of horseshoe crabs collected for bleeding. Biomedical companies should include the potential effects of harvest methods when determining what may be typical stressful conditions for the horseshoe crabs, or they could consider using a harvesting method that would be the least stressful on the animals. All LAL producers can potentially implement some form of these recommendations, thereby altering bleeding process protocols to decrease the stress and mortality levels that these horseshoe crabs experience. Implementation of these recommendations would decrease the impact of the biomedical industry on the potentially declining horseshoe crab population and aid in conservation of this species.

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A study of tagging methods for the sea cucumber *Cucumaria frondosa* in the waters off Maine

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The sea cucumber fishery in waters off Maine is developing and has recently experienced great increases in landings, corresponding to expanding export markets. Between 1994 and 1996, reported landings ranged from one to three million pounds (Fig. 1). In 1999, reported landings were over eight million pounds and rose to over nine million in 2000 (Feindel[1]). Like other developing fisheries, we have little information about the biology and ecology of the sea cucumber off Maine, limited data on the fishery, and little knowledge about the key life history processes that characterize its population dynamics. Therefore, we have a limited understanding of the current status of the resource and the impacts the fishery may have on the stock.

Most research done so far has been done on the red sea cucumber (*Parastichopus californicus*) in the northwestern United States and British Columbia (e.g., Bradbury et al., 1996; Zhou and Shirley, 1996; Phillips and Boutillier, 1998; Perry et al., 1999; Cripps and Campbell, 2000). Limited research has been done along the eastern coast of North America, although some studies have added to our knowledge of *Cucumaria frondosa*. A study of the ecology and behavior of *C. frondosa* was conducted in Maine by Jordan (1972). More recent studies have focused on fertilization success and feeding behavior as it relates to aquaculture (Hamel and Mercier, 1997; Medeiros-Bergen and Miles, 1997; Singh et al., 1998). These studies have improved our knowledge of *C. frondosa* but still do not provide enough information on growth, one of the life history processes most important for understanding the population dynamics of sea cucumbers in waters off Maine.

Because of the lack of hard tissue in sea cucumbers to lay down growth increments, tagging is the method undertaken to determine age. Tagging studies of sea cucumbers are difficult because external tags are frequently lost and internal tags can be shed through the body wall. Sea cucumbers have been tagged *in situ* with limited success (Shelley, 1981; Conand[2]) by using a small T-bar tag that is inserted through the body wall with a tagging gun (Harriott, 1980). Previous studies of manual tags in *P. californicus* in Washington indicated that the presence of these tags had no significant effect on the behavior of the sea cucumbers and did not affect their ability to react to shifts in the salinity of the experimental medium (Fankboner[3]). Typically, necrosis of the tissue surrounding the tag occurs and tags fall out within a few months (Morgan, 2000). Morgan suggested that this method may be adequate for tagging broodstock in captivity over short periods of time and stated that tag loss may be minimized by ensuring that the T-bar is pushed right through the dermis and attempting to make a puncture wound that is as small and clean as possible. Schroeter et al. (2001) used tags to conduct a growth study of *P. parvimensis* in California by following individuals through repeated surveys at two sites. Only 17.6 % of 1224 tagged animals were recaptured over one year. These studies indicate that manual tags may not be an effective means for studying growth because of the high frequency of shedding tags by sea cucumbers.

Previous researchers have also used fluorescent dyes to stain the calcareous plates surrounding the buccal cavity of sea cucumbers but with varied results. Successful staining has depended on the timing of the injection of the dye and the deposition of calcium for growth of the mouthparts (Conand[2]). Other work has suggested that fluorescent marking by dyeing the ossicle of the juveniles with tetracycline may be useful (Tanaka[4]). However, injec-

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tions are time-intensive and marks often become difficult to discern after several months. Also, sea cucumbers could not be located soon after release in some follow-up investigations.

Yanagisawa (1995) successfully used a hot wire to brand the sea cucumber *Stichopus japonicus*. Although most of the marked juveniles could be distinguished three months later, it seemed to be difficult to discern their marks after four months.

The results of previous worldwide sea cucumber tagging studies vary in success by species, investigator, and location. It is also possible that the technique varied significantly for studies involving the same styles of tagging. Therefore, the conclusions of these previous tagging studies may not be applied to the sea cucumber population off Maine. Factors such as dermal thickness and seasonal fluctuations likely alter the capacity of the sea cumcumber to retain tags.

The objective of this study was to ascertain whether a reliable means of tagging the Maine sea cucumber could be identified. Tagged animals would provide information on growth and movement for later studies. This important biological information would be critical for sea cucumber stock assessment and management.

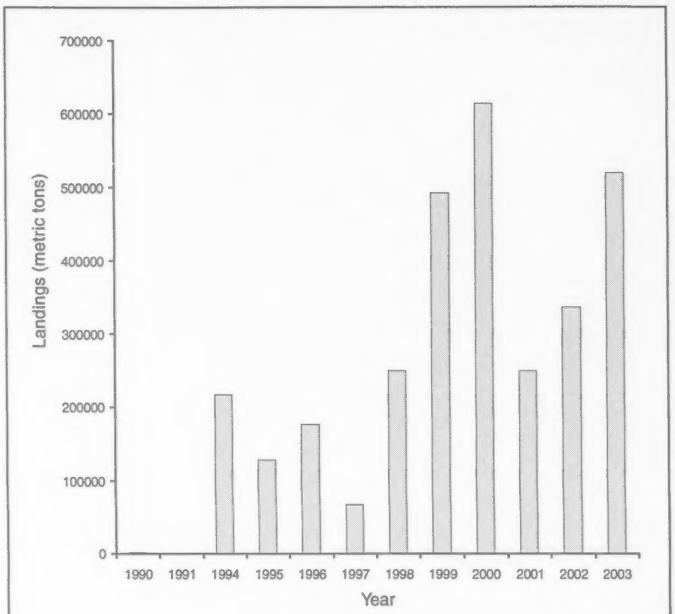

Figure 1

Historical landings (metric tons) for the sea cucumber *Cucumaria frondosa* fishery in Maine.

Materials and methods

The study was carried out between April and September 2003 to determine the effectiveness of various tags. Initially, animals were tested by using 1) long and short double T-bar tags inserted with a tagging gun; 2) dart tags; 3) streamer tags; and 4) cinch-up tags. A control group was also included in which animals were punctured with the tagging gun to observe any mortality from the piercing. It was evident in the first minute that dart tags, streamer tags, and cinch-up tags would not stay within the cucumber body wall and these methods were not considered further.

Next, 5) a rope gun was tested as a means of scarring (branding) animals. Rope guns are normally used to burn through rope by applying the hot tip to the surface of a rope. For tagging purposes, the rope gun tip was applied to the dermis of *C. frondosas* to produce a recognizable scar; the process is similar to that used in branding cattle. Although the rope gun created a noticeable mark, many animals already possessed significant scarring from being dragged during harvesting or from interactions with fishing gear and other marine organisms, and the new scar would not stand out to an observer. The branding method was therefore also discarded.

Finally, 6) liquid orange and yellow fluorescent dyes composed of visible implant elastomer (VIE) were injected into the animals. VIE is a silicone-based material that is mixed immediately before use and quickly becomes a pliable, biocompatible solid. VIE tags were injected internally beneath the epidermal tissue within tube feet, along the body wall, or near the oral cavity. The dye did not spread and all marks were small pin points visible only under ultraviolet light.

Short and long double T-bar tags and fluorescent dyes served as the remaining tagging methods studied during the course of the investigation. Seventy tagged sea cucumbers from the Gulf of Maine were observed over time (Table 1). Ten animals were injected with orange and yellow fluorescent dyes and 45 animals were punctured with long or short T-bar tags. Long tags had a distance of 45 mm between T-bar ends. Short tags had a distance of 19 mm between T-bar ends. Twenty-one tags punctured the body wall just under the muscle so that one end remained inside the animal (single-anchor tags). The remaining 24 passed completely through the body wall twice so that both ends of the T-bar tags were exposed (double-pass tags). Finally, 15 control animals were punctured with the tagging gun, 9 with double punctures and 6 with single punctures. Sea cucumbers were kept in tanks in the laboratories at the Maine Department of Marine Resources in West Boothbay Harbor, Maine. The presence and absence of tags was noted every day from April to September 2003 for each sample. Notation was made when tags were partially in place or no longer observable at all.

Results and discussion

Table 1

Tagging study design for the sea cucumber *Cucumaria frondosa* off Maine. Total numbers sampled for each method are indicated as total animals in the right column. Parentheses identify the number of animals sampled.

Tagging method						Total animals
Fluorescent dyes						10
Double T-bar tags						
Double-pass		Single-anchor				45
Long tag	(9)	Long tag	(6)			
Short tag	(15)	Short tag	(15)			
Control						15
Tagging gun puncture		Double-puncture	(9)	Single-puncture	(6)	
Total						70

The tagging study was successful in providing information on retention rates for the different tagging methods observed during the 140 days of the investigation (Fig. 2). Fluorescent dye injections and single-anchor long T-bar tags were the most successful means identified in tagging sea cucumber in the waters off Maine. Eighty percent with dye continued to fluoresce under the UV light and 65% of single-anchor long T-bar tags remained embedded in the sea cucumbers at the end of the investigation on day 140. The T-bar method may be the most practical for short-term research on this species considering the time and expense of fluorescent dye injection. Another advantage over injections is that the tagging gun technique is much more easily repeatable among subjects, whereas the site of injection varied among animals depending on the location of soft tissue.

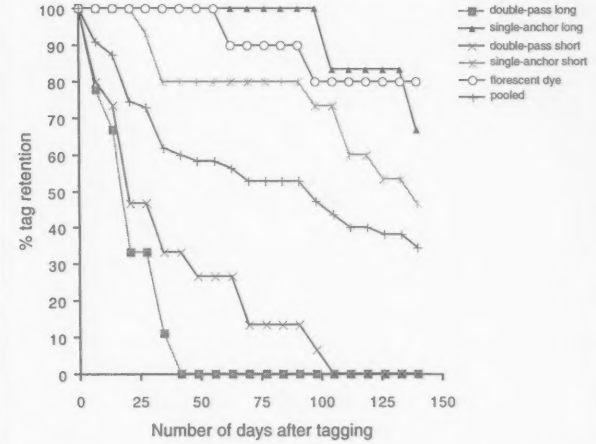

Figure 2

Tagging retention in the sea cucumber *Cucumaria frondosa* for individual-style tags over 140 days of observation in the waters off Maine. Note that florescent dye and single-anchor long T-bar tags were the most successful methods identified.

Double-pass T-bar tags were expelled faster than those in the single-anchor condition. With the double-pass tag, it appeared that the long portion of the tag under the animal's epidermis gradually moved upward until it was expelled outside of the skin without scarring the animal. The longer bars were pushed out the fastest and all were shed before day 50. Shorter T-bar tags took almost twice as long to reach 0% retention as the longer T-bars in the double-pass condition, perhaps because the shorter bars pinched the dermis tightly between tag bars, whereas animals with the longer bars were able to relax their entire body wall. After tags were shed in all T-bar conditions, it was not possible to tell which experimental condition each individual had experienced. No mortality was observed from the tags or from the control punctures.

Five T-bar tagged animals experienced a punctured respiratory tree caused by the tagging gun. The respiratory tree is an internal organ used for breathing by pumping seawater in and out. After the initial puncture, no trace of the injury could be detected and no differences in appearance or behavior were noted throughout the investigation. Although it was not possible to track which individuals had a partially punctured respiratory tree, injured individuals may have shed tags at a different rate than others in their cohorts. Internal injuries may also have affected the results of later studies of growth and movement with the use of manual tags. With the use of a largest possible sample size and careful placement of the tagging gun, this potential bias can be minimized. Additionally, it is important to consider that tag retention rate in the field may vary more than results observed in the laboratory.

We believe that the fluorescent dye injections and single-anchor long-T-bar tagging methods in our investigation will likely prove effective for a longer-term study with additional preliminary tagging analysis. However, a more effective way to study the growth of sea cucumbers may be the combination of caging and tagging, where tagged individuals of similar size are put in the same cage to monitor a change in size.

Future investigations will test different locations for, and methods of, injecting fluorescent dye and single-anchor long T-bar tags and will help to further identify the best tagging method. When the most successful method is determined, a long-term study will allow us to understand how individual animals grow, move, and change both seasonally and annually.

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Parameterizing probabilities for estimating age-composition distributions for mixture models

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When estimating parameters that constitute a discrete probability distribution $\{p_j\}$, it is difficult to determine how constraints should be made to guarantee that the estimated parameters $\{\hat{p}_j\}$ constitute a probability distribution (i.e., $\hat{p}_j>0$, $\Sigma\hat{p}_j=1$). For age distributions estimated from mixtures of length-at-age distributions, the EM (expectation-maximization) algorithm (Hasselblad, 1966; Hoenig and Heisey, 1987; Kimura and Chikuni, 1987), restricted least squares (Clark, 1981), and weak quasisolutions (Troynikov, 2004) have all been used. Each of these methods appears to guarantee that the estimated distribution will be a true probability distribution with all categories greater than or equal to zero and with individual probabilities that sum to one. In addition, all these methods appear to provide a theoretical basis for solutions that will be either maximum-likelihood estimates or at least convergent to a probability distribution.

However, all these methods are presented in a theoretical context that is useful in understanding the theory, but may not be suitable for actual application. Currently, most modelers will have an optimization program available. This note describes how, in a brief amount of time, the modeler can add a mixture model program to his collection of readily available programs—one that will estimate maximum-likelihood estimates for the mixture problem and will incorporate the experimenter's familiar optimization program.

To do this it is necessary to parameterize the estimated probabilities so that they are in fact guaranteed to constitute a probability distribution (i.e., $\hat{p}_j>0$, $\Sigma\hat{p}_j=1$). Two such parameterizations are the multinomial logit and a parameterization method described by Heifetz and Fujioka (1991). The trick with both parameterizations is that although the parameters that are actually estimated are unconstrained, these unconstrained estimates can be easily transformed to constrained probability estimates.

Materials and methods

Multinomial logit parameterization

Consider parameterizing the probabilities using the classic logit model:

$$p_j = \frac{r_j}{(1+\sum r_k)} \quad j,k=1,\ldots,a-1 \text{ and}$$

$$p_a = \frac{1}{(1+\sum r_k)}.$$

The r_j can be guaranteed to be positive by defining $r_j=\exp(x_j)$. The parameters that are estimated are $x_j=\ln(r_j)$. In turn the $\{x_j\}$ are used to estimate the $\{r_j\}$, and then the $\{p_j\}$. Thus, $x_1, \ldots, x_{a-1}$ if are estimated on $(-\infty, \infty)$, the resulting $\{\hat{p}_j\}$ is guaranteed to be a probability distribution. The relationship between the $\{p_j\}$ and $\{r_j\}$ is a unique one-to-one mapping because given any $\{p_j\}$, $r_j=p_j/p_a$, for $j=1, \ldots, a-1$.

Heifetz and Fujioka parameterization

Heifetz and Fujioka (1991), for a tagged fish migration problem, used a somewhat similar parameterization that guaranteed estimated parameters would be probability distributions. Suppose a distribution consisted of categories with probabilities $\{p_j\}$. The Heifetz-Fujioka (H-F) parameterization would be

$$p_j = \frac{r_j}{\sum r_k}\left(1-\exp(-\sum r_k)\right)$$

$$j,k=1,\ldots,a-1 \text{ and}$$

$$p_a = \exp(-\sum r_k)$$

$$\text{where } r_k>0 \text{ for } k=1,\ldots,a-1.$$

As above r_j, can be guaranteed to be positive by defining $r_j=\exp(x_j)$ and the parameters that are estimated are $x_j=\ln(r_j)$. Also as above, $\{x_j\}$ are used to estimate the $\{r_j\}$, and then the $\{p_j\}$. Thus, if $x_1, \ldots, x_{a-1}$ are estimated on $(-\infty, \infty)$, the resulting $\{\hat{p}_j\}$ is guaranteed to be a probability distribution. The relationship between the $\{p_j\}$ and $\{r_j\}$ is a unique one-to-one mapping because given any $\{p_j\}$, $r_j=-p_j\ln(p_a)/(1-p_a)$, for $j=1, \ldots, a-1$.

H-F probabilities as exploitation rates

Stock assessment modelers would recognize the formula

$$p_j = \frac{r_j}{\sum r_k}\left(1-\exp(-\sum r_k)\right)$$

as the exploitation rate formula where p_j takes the role of the exploitation rate (u_j) and r_j takes the role of the instantaneous mortality rate F_j. The formula $r_j=-p_j\ln(p_a)/(1-p_a)$ indicates that the instantaneous mortality rate can be solved in closed form, but this is generally not true because the natural mortality rate M is generally known as an instantaneous mortality rate rather than an exploitation rate.

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However, when exploitation rates are all known, as in some ecological modeling exercises (e.g., $u_j=C_j/N_j$, i.e., catch per population), then all instantaneous mortality rates can be solved in closed form.

Example: As an example, the logit and H-F parameterization can be used to estimate the distribution mixture problem for empirical length-at-age data. To describe the mixture problem for empirical length-at-age data, let

$i = 1, \ldots, n$ be the length category index;
$j = 1, \ldots, a$ be the age category index;

f_i = the observed length frequency, for which we wish to estimate the corresponding age distribution;
$\bar{f}_i = f_i/f$, where $f=\Sigma f_i$, the observed length distribution;
p_j = the unknown age distribution we wish to estimate from the observed length distribution;
q_{ij}=Prob$[i|j]$ = the observed distribution of length at age, estimated from age-length data;
$l_i = \Sigma_j p_j q_{ij}$ = length distribution estimated from an age distribution and observed length-at-age distributions.

Solutions to the empirical distribution mixture problem can be stated as solving for that age distribution $\{p_j\}$, which when combined with length i at age j, say $\{q_{ij}\}$, provides the "best" weighting to reproduce some length frequency $\{\bar{f}_i\}$. "Best" may be defined as

1 Maximum likelihood (Kimura and Chikuni, 1987):

$$L = const. + \sum_{i=1}^{n} f_i \log(l_i).$$

2 Minimum chi-square

$$\chi^2 = \sum_{i=1}^{n} (f_i - fl_i)^2 / (fl_i).$$

For both of these estimation problems, the estimated $\{\hat{p}_j\}$ distribution must be constrained to sum to one. The logit or H-F parameterization simplifies and unifies the estimation of $\{p_j\}$ for these or any other objective function. Any multivariate function optimization program, using these parameterizations, can generate estimated probability distributions whose components are positive and will be guaranteed to sum to one. Therefore any multivariate optimization program with these parameterizations can be used to estimate $\{p_j\}$.

Results

Greenland turbot (*Reinhardtius hippoglossoides*) length-at-age data were originally used to illustrate the iterated age-length key (IALK) (Kimura and Chikuni, 1987).

Table 1

Age distributions for Greenland turbot (*Reinhardtius hippoglossoides*) estimated by fitting length at age data to 1983 length-frequency data (Kimura and Chikuni, 1987). Except for the iterated age-length key (IALK) algorithm, all estimates were arrived at by using the logit or Heifetz-Fujioka (H-F) parameterizations which provided identical results.

Age (yr)	Maximum likelihood	IALK algorithm	Minimum χ^2
4	0.0353	0.0353	0.0377
5	0.1903	0.1903	0.1858
6	0.2281	0.2281	0.2229
7	0.1291	0.1291	0.1318
8	0.1125	0.1125	0.1117
9	0.0380	0.0380	0.0349
10	0.0525	0.0525	0.0554
11	0.0000	0.0000	0.0000
12	0.0332	0.0332	0.0383
13	0.0023	0.0023	0.0000
14	0.0204	0.0204	0.0235
15	0.0168	0.0168	0.0162
16	0.0289	0.0289	0.0248
17	0.0253	0.0253	0.0218
18	0.0680	0.0680	0.0719
19	0.0042	0.0042	0.0036
20	0.0152	0.0152	0.0198

We used the 1983 length-frequency data from that data set, along with the length-at-age data, to illustrate the methods of estimating mixture probabilities with the logit and H-F parameterizations (Table 1). Except for results from the IALK algorithm, all parameters were estimated by using an optimization program with the logit and H-F parameterizations (i.e., the latter two parameterizations gave identical results). Results also showed that maximum-likelihood estimates from either the logit or H-F parameterizations provided maximum-likelihood estimates identical to those estimated by using the IALK algorithm.

Discussion

It is probable that the logit and H-F parameterizations would provide nearly identical solutions for a given data set and objective function. If maximum likelihood solutions are unique, either parameterization should provide the maximum-likelihood estimates because the reparameterizations of probabilities are one-one mappings that lead to invariance properties when optimization is performed. The maximum-likelihood solutions will generally be unique when all $\hat{p}_j>0$ (Kimura and Chikuni, 1987). However, difficulties in searching in multivariate spaces, and limited computational precision may cause differences in the estimates.

Simple parameterizations, like direct estimates of $\{p_j\}$, will allow solutions that have negative probabilities. Constrained solutions, which allow maximum likelihood estimates and other types of estimates, will generally make these negative components of the probability distribution have zero values. Such solutions are boundary value solutions and may not be unique (Kimura and Chikuni, 1987). This is another reason it is difficult to claim unique solutions for the mixture problem.

From the modeling perspective, we illustrate the usefulness of reparameterization to impose mathematical constraints. In the context of the mixture problem the suggested parameterizations are reasonably transparent and allow the modeler to use familiar software. The reason we propose the methods described in this note is not that these methods provide superior estimates to those described in the literature, but that the procedure for estimation may actually be more straightforward and transparent for modelers more interested in solutions than in theory.

Because of its simplicity, effectiveness, and ready applicability to different objective functions, modelers may prefer optimization using the logit or H-F parameterizations to estimate probability distributions for the mixture problem. Another advantage of these reparameterizations is that they can be more generally applied, for example, to estimate geographic distribution in migration models (Heifetz and Fujioka, 1991; Shimada and Kimura, 1994).

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Morphometric differentiation in small juveniles of the pink spotted shrimp (*Farfantepenaeus brasiliensis*) and the southern pink shrimp (*F. notialis*) in the Yucatan Peninsula, Mexico

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The morphometric and morphological characters of the rostrum have been widely used to identify penaeid shrimp species (Heales et al., 1985; Dall et al., 1990; Pendrey et al., 1999). In this setting, one of the constraints in studies of penaeid shrimp populations has been the uncertainty in the identification of early life history stages, especially in coastal nursery habitats, where recruits and juveniles dominate the population (Dall et al., 1990; Pérez-Castañeda and Defeo, 2001). In the western Atlantic Ocean, Pérez-Farfante (1969, 1970, 1971a) described diagnostic characters of the genus *Farfantepenaeus* that allowed identification of individuals in the range of 8–20 mm CL (carapace length) on the basis of the following morphological features: 1) changes in the structure of the petasma and thelycum; 2) absence or presence of distomarginal spines in the ventral costa of the petasma; 3) the ratio between the keel height and the sulcus width of the sixth abdominal somite; 4) the shape and position of the rostrum with respect to the segments and flagellum of the antennule; and 5) the ratio between rostrum length (RL) and carapace length (RL/CL). In addition, she classified *Farfantepenaeus* into two groups according to the shape and position of the rostrum with respect to the segments and flagellum of the antennule and the ratio RL/CL: 1) *F. duorarum* and *F. notialis*: short rostrum, straight distally, and the proximodorsal margin convex, usually extending anteriorly to the end of distal antennular segment, sometimes reaching to proximal one-fourth of broadened portion of lateral antennular flagellum, with RL/CL <0.75; and 2) *F. aztecus*, *F. brasiliensis*, *F. paulensis*, and *F. subtilis*: long rostrum, usually almost straight along the entire length, extending anteriorly beyond the distal antennular segment, sometimes reaching to the distal one-third of broadened portion of lateral antennular flagellum, with RL/CL >0.80. Pérez-Farfante stressed that, for the recognition to species level of juveniles <10 mm CL, all the characters listed above should be considered because occasionally one alone may not prove to be diagnostic. However, the only characters that could be distinguished for small juveniles in the range 4–8 mm CL are those defined on the rostrum. Therefore, it has been almost impossible to identify and separate small specimens of *Farfantepenaeus* (Pérez-Farfante, 1970, 1971a; Pérez-Farfante and Kensley, 1997).

Pink spotted shrimp (*F. brasiliensis*) and southern pink shrimp (*F. notialis*) have a wide geographic distribution in coastal environments of the Atlantic Ocean. The pink spotted shrimp is distributed within the western Atlantic from Cape Hatteras, North Carolina, to Cabo Frio, Brazil (22°00′S, 42°00′W: Pérez-Farfante, 1969), including the southwestern Gulf of Mexico and the Caribbean coasts of Mexico (Pérez-Farfante, 1971b). The southern pink shrimp ranges from the northwestern Yucatan Peninsula, Mexico (20°45′N, 90°25′W: Pérez-Castañeda and Defeo, 2000), to Rio de Janeiro (Brazil) in the western Atlantic, including Cuba and the Virgin Islands in the Caribbean Sea. The species is also reported from Mauritania to Angola in the eastern Atlantic Ocean (Pérez-Farfante, 1969; Pérez-Farfante and Kensley, 1997).

The current identification keys show that, for shrimps ≥8 mm CL, the pink spotted shrimp has a proportionately longer rostrum than the southern pink shrimp. However, there are no scientific data to separate these species at individual sizes <8 mm CL (Pérez-Farfante, 1970, 1971a). This is particularly important where both penaeid shrimps are found in the same region, mainly between the Yucatan Peninsula and along the Caribbean coast of Mexico, Puerto Rico, and Colombia. In this geographic region, small juveniles of both species have been almost impossible to separate and have been classified only to genus level (Stoner and Zimmerman, 1998; May-Kú, 1999; Pérez-Castañeda and Defeo, 2001; Criales et al., 2002). In this note we provide quantitative and qualitative information that allows separation of the sympatric *F. brasiliensis* and *F. notialis* in the range of 4–8 mm CL based on shrimp collected in the Río Lagartos and Yalahau coastal lagoons of the Yucatan Peninsula, Mexico.

Materials and methods

Shrimp were collected by using a Renfro beam trawl (1.6×0.5 m mouth, 1.5 m total length, and 1.0-mm mesh), which was hand hauled in two coastal lagoons of northeastern Yucatan Peninsula (Fig. 1). The Río Lagartos

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lagoon (21°26′–21°38′N, 87°30′–88°15′W) was sampled from November 1996 to April 1997, whereas the Yalahau lagoon (21°26′–21°36′N, 87°08′–87°29′W) was sampled from June 2001 to May 2002. These coastal lagoons are considered the main nursery areas for *F. brasiliensis*, accounting for 80–95% of the total shrimp abundance, followed by *F. notialis* (May-Kú, 1999; May-Kú and Ordóñez-López, 2000). Shrimps (4–10 mm CL) were preserved in 10% formaldehyde, prior to examination. Rostrum length (RL; distance from the tip of the rostrum to the postorbital margin of the carapace) and carapace length (CL; distance from the postorbital margin to the posterior margin of the carapace) were measured with an ocular micrometer to the nearest 0.1 mm. Furthermore, the shape and position of the rostrum with respect to the segments and flagellum of the antennule were considered (Pérez-Farfante, 1969; 1970, 1971a). Shrimps ranging from 8 to 10 mm CL were identified to species level according to the characters outlined by Pérez-Farfante (1970, 1971a). The relationship between rostrum length and carapace length (RL-CL) was determined for each species (*F. brasiliensis*, *F. notialis*) in each lagoon, and fitted to the linear function $RL = a + b\ CL$, where a and b are coefficients. One-way analysis of covariance (ANCOVA) was used to evaluate differences between species in the RL-CL relationship for each site, with RL as the dependent variable and CL as the covariate. Assumptions of homoscedasticity and homogeneity of slopes (parallelism) were met after log-transformation of RL. Lastly, the RL/CL ratio was estimated to remove the differences in individual sizes between species, then plotted against CL and determined for each lagoon. A one-way analysis of variance (ANOVA) was conducted to test interspecific differences between mean RL/CL in each lagoon. The original RL/CL values were log-transformed to fulfill ANOVA assumptions.

Results

A total of 13,234 shrimps in the size range 4–10 mm CL were collected. Of the 727 organisms measured in the Río Lagartos lagoon, 74.6% were *F. brasiliensis* and 25.4% *F. notialis*. In Yalahau lagoon, from the 12,507 organisms measured, 91.7% were *F. brasiliensis* and 8.3% *F. notialis*. In the two lagoons, both species showed a significant positive linear RL-CL relationship ($r^2 \geq 0.92$; $P<0.001$). Significant differences were detected between the species (ANCOVA: $P<0.001$; Fig. 2; Table 1)—*F. brasiliensis* having a longer rostrum than *F. notialis* for a given CL.

The RL/CL ratio for both species ranged from 0.42 to 0.94. In both lagoons the scatter plots

Figure 1

Location of Río Lagartos and Yalahau lagoons off the Yucatan Peninsula. The distribution of *Farfantepenaeus brasiliensis* and *F. notialis* along the Mexican coast of the Gulf of Mexico and Caribbean Sea is indicated by oblique lines.

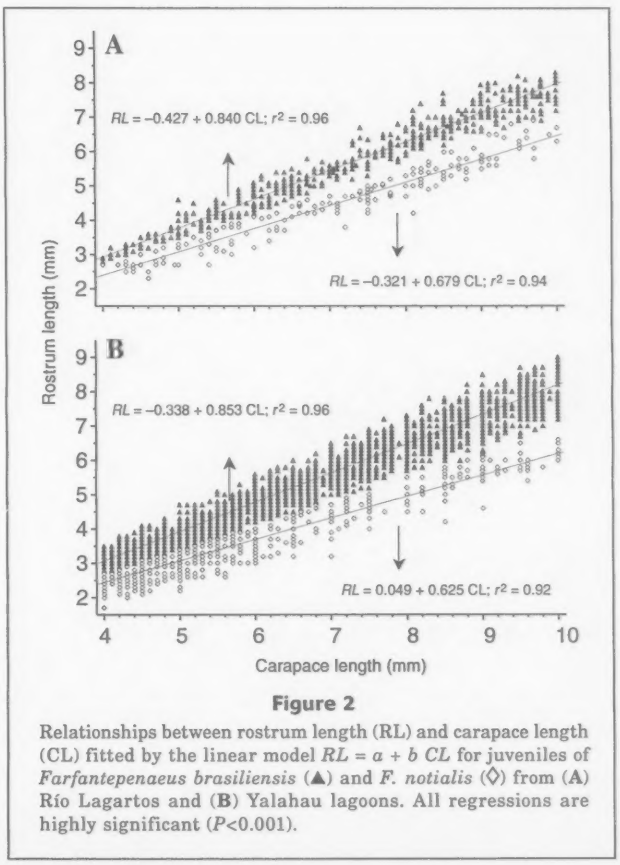

Figure 2

Relationships between rostrum length (RL) and carapace length (CL) fitted by the linear model $RL = a + b\ CL$ for juveniles of *Farfantepenaeus brasiliensis* (▲) and *F. notialis* (◇) from (**A**) Río Lagartos and (**B**) Yalahau lagoons. All regressions are highly significant ($P<0.001$).

Table 1

Parameters of the linear regression model between rostrum length (RL) and carapace length (CL) fitted for *Farfantepenaeus brasiliensis* and *F. notialis*, in the size range of 4–10 mm CL, for Río Lagartos and Yalahau. ANCOVA results (log-transformed RL) are also shown, including tests of homogeneity of slopes and differences in RL at CL (main effect) between species. "*a*" and "*b*" represent, respectively, the intercept and slope of the regression lines. SE = standard error; ns = not significant; *** = $P<0.001$.

	a Mean (SE)	*b* Mean (SE)	r^2	*n*	*P*
Río Lagartos					
F. brasiliensis	−0.427 (0.055)***	0.840 (0.007)***	0.96	542	***
F. notialis	−0.321 (0.091)***	0.679 (0.013)***	0.94	185	***
ANCOVA					
Homogeneity of slopes: $F_{1,723}=1.212$; $P=0.271$					
Main effect: $F_{1,724}=1{,}430.2$; $P=0.00001$					
Yalahau					
F. brasiliensis	−0.338 (0.010)***	0.853 (0.002)***	0.96	11,467	***
F. notialis	0.049 (0.032) ns	0.625 (0.006)***	0.92	1040	***
ANCOVA					
Homogeneity of slopes: $F_{1,12503}=0.966$; $P=0.326$					
Main effect: $F_{1,12504}=12486.4$; $P=0.0001$					

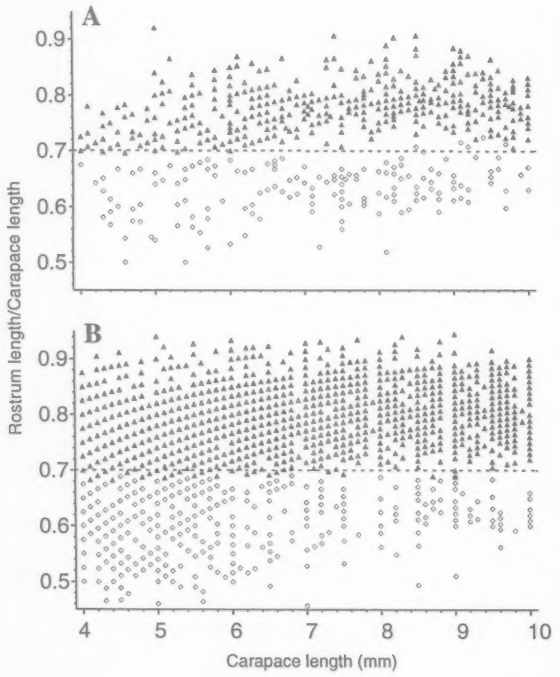

Figure 3

Scatter plot of rostrum length/carapace length (RL/CL) ratios against CL for juveniles of *Farfantepenaeus brasiliensis* (▲) and *F. notialis* (◊) from (**A**) Río Lagartos and (**B**) Yalahau lagoons. The separation between species at RL/CL = 0.70 is indicated by a dotted line.

of RL/CL ratios against CL by species fell into two distinct clouds representing dissimilar morphometric characteristics of the species, with the point of separation between species set at a value of 0.70 (Fig. 3). The RL/CL ratios were significantly higher in *F. brasiliensis* than in *F. notialis*, both for Río Lagartos (ANOVA, $F_{1,725}=1778.33$, $P<0.001$) and Yalahau (ANOVA $F_{1,12505}=16627.64$, $P<0.001$). For Río Lagartos, *F. brasiliensis* had a mean RL/CL ratio ±SD of 0.78 ±0.04 and a range of 0.70–0.92, whereas the mean RL/CL ratio in *F. notialis* was 0.63 ±0.04, with a range of 0.50–0.72. In Yalahau lagoon, the RL/CL ratio in *F. brasiliensis* was 0.80 ±0.05 (range 0.68–0.94), whereas in *F. notialis* these values were 0.62 ±0.05 (0.43–0.69; Fig. 4). The above values indicate a morphometric divergence between the two species, with higher values corresponding to *F. brasiliensis* and lower ones to *F. notialis*.

Small juveniles presented clear differences between species in the shape and position of the rostrum with respect to the segments and the flagellum of the antennule. *Farfantepeaeus brasiliensis* had a rostrum that was straight and slender, extending beyond the 3rd antennular segment, and generally reaching the broadened portion of lateral flagellum (Fig. 5A), whereas *F. notialis* had a lightly convex rostrum, never extending beyond the third antennular segment (Fig. 5B).

Discussion

We found clear morphometrical and morphological rostrum differences between small juveniles

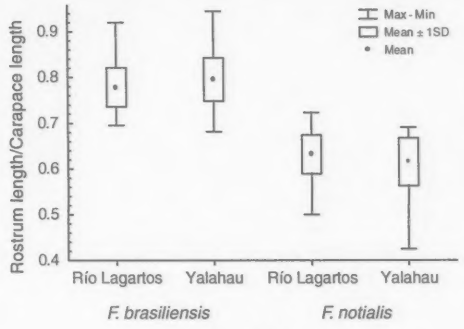

Figure 4

Box and whisker plots of the rostrum length/carapace length (RL/CL) ratios for *Farfantepenaeus brasiliensis* and *F. notialis*, discriminated by lagoon.

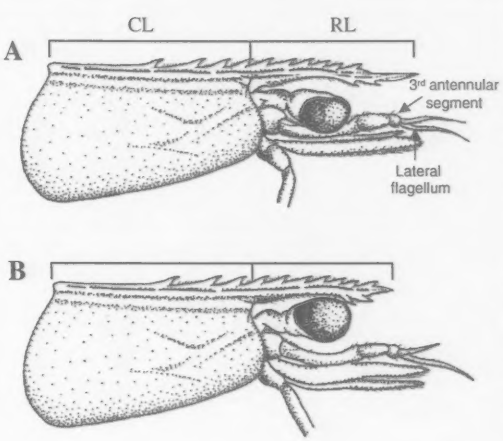

Figure 5

Distinguishing features of the shape of the rostrum and its position with respect to the segments and the flagellum of the antennule in *Farfantepenaeus brasiliensis* (**A**) and *F. notialis* (**B**). CL = carapace length, RL = rostrum length.

(4–8 mm CL) of *F. brasiliensis* and *F. notialis* in two coastal lagoons of the Gulf of Mexico. Our results also complement those obtained by Pérez-Farfante (1969; 1970, 1971a) because the unique characters of the rostrum highlighted by that author for juveniles ≥8 mm CL persisted in our study for small juveniles (<8 mm CL). We also found interspecific differences in the growth patterns given by the RL-CL relationship (slopes) and in the RL/CL ratio (intercept differences). These results have important implications. Dall et al. (1990) mentioned that the shape and increase in size of different body structures in crustaceans depend on the species, age, sex, and the surrounding environment. Our comparison of the regression lines between RL and CL of the two shrimp species analyzed in the present study, coming from Yalahau and Río Lagartos lagoons, showed unambiguously that the growth pattern, or degree of change in RL in relation to CL, differed between the two species at all sizes of the specimens. Different intercepts, on the other hand, implied that although the growth patterns may be the same over the size range measured (regression lines are almost parallel), the relative body proportions of both species are different. These differences are likely due to different growth rates at some earlier size (Haddon and Willis, 1995). Both types of difference imply that the relative proportions of the body, or the body shape, are distinct for the species analyzed (Haddon and Willis, 1995). An analysis of the RL-CL relationship revealed that, at a given CL, the RL in *F. brasiliensis* grows 1.23–1.29 times faster than in *F. notialis*. On the other hand, the marked differences in the RL/CL ratio imply that this morphometric character is species-specific and, at a given CL, the pink spotted shrimp has a consistently longer rostrum than southern pink shrimp, with a value of 0.70 as a point of separation between species. These results are also qualitatively observed in the shape and position of the rostrum with respect to the segments and the flagellum of the antennule in both species.

In conclusion, we successfully separated small juveniles of two shrimp species that are found in coastal lagoons of the Gulf of Mexico. Both species can be identified by easily observed and quantified rostrum characteristics.

Acknowledgments

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Exploring intraspecific life history patterns in sharks

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Marine ecosystems compose the major source (85%) of world fisheries production (Garcia and Newton, 1997). Although only a few fish species tend to dominate fishery catches (Jennings et al., 2001), a large diversity of fishes representing varied taxonomic levels, ecological guilds, and life histories is commonly taken. Recently, 66% of global marine resources were determined to be either fully, heavily, or over-exploited (Botsford et al., 1997). Considering the current state of many fisheries, the large diversity of species taken globally, and the general lack of resources to adequately assess many stocks, it has become important to develop shortcuts that may provide methods fisheries scientists can use to determine which stocks are in danger of overexploitation and which recovery plans are appropriate when biological data are limited (Stobutzki et al., 2001).

Applications of life history theory have proven a potentially useful means to accomplish such tasks (Stearns, 1992; Reynolds et al., 2001). Life history traits such as maximum size and age, maturity, mortality, and growth are correlated among teleost fishes (Adams, 1980; Winemiller and Rose, 1992; Gunderson, 1997; Cortés, 2000) and the relationships among such traits can be used to infer some general life history patterns. These general patterns reveal that teleost fishes with higher maximum ages tend to be larger, mature later, grow more slowly, and have lower natural mortality rates (K-selected species, Adams, 1980), whereas teleost fishes with lower maximum ages tend to show the opposite relationships (r-selected species, Adams, 1980). Correlations among traits may also allow one to approximate difficult to measure life history traits from traits that are easier to measure and possibly anticipate response to exploitation rates where life history data are limited (Jennings et al., 1999).

Applying these patterns to fisheries trends reveals some consistent and useful explanations. Jennings et al. (1998) found that teleost fishes from the northeast Atlantic that have decreased in abundance are generally K-selected species. Jennings et al. (1999) demonstrated that tropical teleost fishes of greatest maximum sizes were most vulnerable to exploitation. And Rochet (2000) illustrated the limitations of life history plasticity to compensate for heavy fishing pressure among four orders of teleosts.

Elasmobranchs, and particularly sharks, have also shown life history patterns similar to those of teleosts (Cortés, 2000; Frisk et al., 2001). Cortés (2000) offered three general life history patterns for sharks: 1) large litters, moderate to high longevity, large size, small offspring, slow growth, 2) small litters, high longevity, large size, large offspring, slow growth and 3) small litters, low longevity, small size, small offspring, fast growth. Simplified applications of the life history patterns have also been applied to elasmobranch fisheries. Smith et al. (1998) demonstrated that larger, later-to-mature Pacific shark species have lower rebound potentials (i.e., abilities to recover from fishing pressure), whereas Frisk et al. (2001) showed a similar pattern in sharks and rays in the north Atlantic. These relationships have been recommended as particularly useful when managing data-poor elasmobranch species (Musick et al., 2000).

As indicated by the above studies, variation in life history traits and patterns among shark species is well established (Cortés, 2000) and such relationships may be useful for the management of these fishes, but it is not known how these relationships may change within a species. Specifically, if and how do intraspecific life history traits of cosmopolitan species vary in different areas of the world? The spiny dogfish (*Squalus acanthias*) provides an alluring preamble to the topic: northeast Pacific spiny dogfish have been aged to 80+ years, and females mature at around 35 years (Jones and Geen, 1977; Saunders and McFarlane, 1993), whereas spiny dogfish in the north Atlantic obtain a maximum age of about 40 years, maturing at 12 years (Rago et al., 1998).

In the present study, I used generalized linear models (GLMs) to investigate whether spatial differences in life history traits, such as those seen in the spiny dogfish, reveal consistent patterns when compared with other spatially resolved life history information from other shark species. I then, as demonstration of potential utility, applied these models to predict life history trait values for areas lacking information for two species of shark, spiny dogfish (*S. acanthias*) and blue shark (*Prionace glauca*).

Materials and methods

Information for five life history traits (age at maturity, longevity (maximum age), mean fecundity, maximum size, and size at maturity) from 17 shark species in six families (Appendix, Table 1) for seven general areas (North Pacific (NP); North Atlantic (NoA); Gulf of Mexico (GM); Indian Ocean (I); Central Pacific (CP); South Pacific (SP); South Atlantic (SA)) was extracted from three primary literature sources (Smith et al., 1998; Cortés, 2000, 2002). Area distinctions were based on those by Cortés

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Fish. Bull. 104:311–320 (2006).

Table 1

List of shark species used in the analyses. Species abbreviations are used in tables and figures to simplify presentation.

Scientific name	Common name	Abbreviation
Carcharhinidae		
Carcharinus acronotus	Blacknose shark	Cac
Carcharinus amblyrhynchos	Grey reef shark	Cal
Carcharinus falciformis	Silky shark	Cfa
Carcharinus leucas	Bull shark	Cle
Carcharinus limbatus	Blacktip shark	Cli
Carcharinus longimanus	Oceanic whitetip shark	Clo
Carcharinus obscurus	Dusky shark	Cob
Carcharinus plumbeus	Sandbar shark	Cpl
Galeocerdo cuvier	Tiger shark	Gcu
Prionace glauca	Blue shark	Pgl
Rhizoprionodon taylori	Australian sharpnose shark	Rta
Sphyrnidae		
Sphyrna lewini	Scalloped hammerhead shark	Sle
Triakidae		
Galeorhinus galeus	Soupfin shark	Gga
Lamnidae		
Isurus oxyrinchus	Mako shark	Iox
Alophiidae		
Alopias superciliosus	Bigeye thresher shark	Asu
Squatinidae		
Squalus acanthias	Spiny dogfish	Sac
Squalus mitsukurii	Shortspine spurdog	Smi

(2000). Spatial resolution to the particular ocean basins in this study was defined by the information I was able to obtain, as were the choice of species and life history traits to analyze. Although other information for these and other species may be currently available, I limited my data to those found in primary peer-reviewed literature. When mean fecundity values were unavailable, the mean was assumed to be the middle value of the fecundity range given. Phylogenetic variance was controlled because I strictly evaluated intraspecific comparisons.

Initial data exploration was performed by visualizing intraspecific gender-based pairwise comparisons by area with dot plots (Fig. 1). Within each species and gender, the outcome of each comparison (i.e., the value for a particular trait in one area greater than, less than, or equal to that of another area) was evaluated. An overall relationship among areas for each life history trait was then constructed as a composite of each pairwise result. The purpose of this exercise was to visually explore the data and assess whether any intraspecific life history patterns by area were apparent.

A GLM framework was then used to construct simple models that quantitatively relate the effect of certain factors (e.g., area, gender, or taxonomic level) to a response variable—in this case to a particular life history trait. The flexibility of the GLM framework also allows one to consider non-normal response distributions while maintaining the advantages of linear regression (Venables and Ripley, 2002) by means of a link function relating the response variable mean to the linear predictors. I had no *a priori* knowledge of the variance structure for each life history trait (some of which will not have variance, given that they are maximum recorded values), so both lognormal (with an identity link) and gamma (with a log link) distributions were considered because of their appropriateness to continuous and nonzero data. Akaike's information criterion (AIC; Burnham and Anderson, 2002) was used to select among models, with the lowest AIC value indicative of the most appropriate model among all considered. Models explored included all combinations of the following factors: area, gender, and the taxonomic levels of species, genus, or family. Models that included the interaction between gender and area and gender and taxonomic level were also considered. Area effects and predictions of life history values among models with the use of the lognormal and gamma distributions were similar, but models with gamma error structure resulted in the lowest standard errors for area effects; thus a gamma error structure was ultimately chosen for each model.

Resultant model effects were used to compare area effects and to predict species- and gender-specific life history trait values for each area. The predicting models were then applied to two species (*S. acanth-*

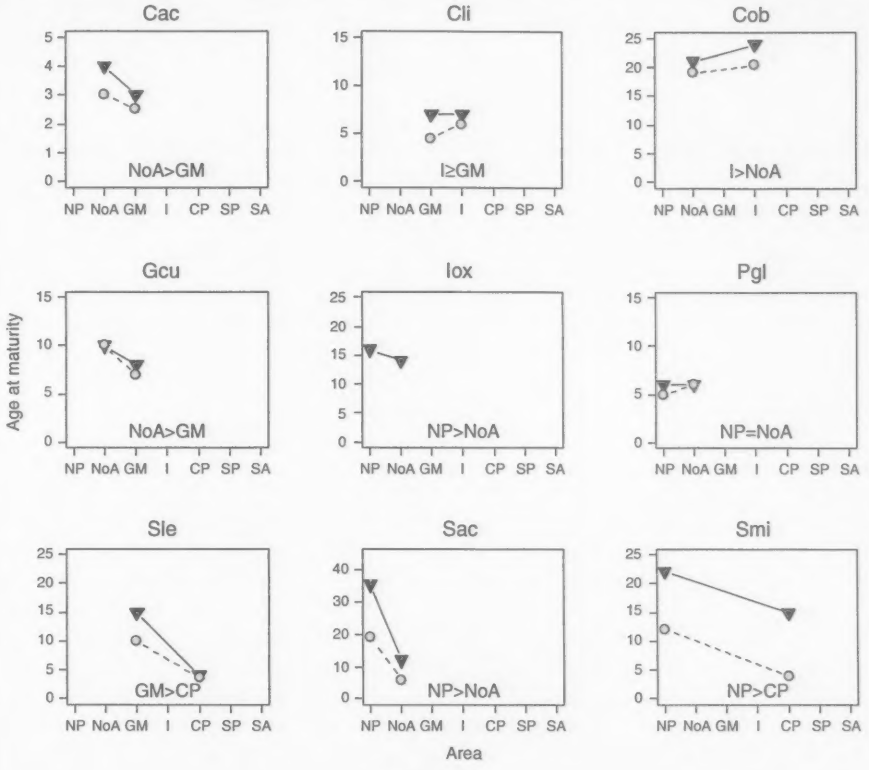

Figure 1

Example of plots used to evaluate the pairwise life history trait (*y*-axis) comparisons among areas (*x*-axis) by species (indicated by the abbreviation at the top of each panel). Gender-specific age at maturity (solid triangles=females; solid circles=males) is illustrated in this example, and the qualitative outcome of the pairwise comparisons is provided within each panel. A key to the species abbreviated names is found in Table 1. Areas: NP=North Pacific; NoA=North Atlantic; GM=Gulf of Mexico; I=Indian Ocean; CP=Central Pacific; SP=South Pacific; SA=South Atlantic.

ias and *P. glauca*) to demonstrate the calculation of life history trait values for areas with missing values. These species were chosen as examples because they are taxonomically different, are found in most of the area designations, and are represented by at least one pairwise comparison for each life history trait. Further investigation of the predictive capacity of these models to fecundity, size at maturity, and maximum size for the two species was performed by cross-validation: for each life history trait, *S. acanthias* or *P. glauca* data for one area were removed, the models were re-estimated, and predicted values for the newly missing area were calculated. Model fits to age at maturity, longevity, and male *P. glauca* size at maturity were not explored with cross validation because these data lacked the sufficient sample size needed to calculate the species effect once one area was removed (at least two remaining areas were needed).

Results

Model structure

For each life history trait that contained gender as a factor, the following final GLM model with a gamma distribution and a log link was selected (i.e., had the lowest AIC value):

$$\ln y_{A+sp\times g} = \ln \beta_A + \ln \beta_g + \ln \beta_{sp} + \ln \beta_{g\times sp}, \quad (1)$$

where β_A = area effect;
β_g = gender effect;
β_{sp} = species effect;
$\beta_{g\times sp}$ = gender and species interaction effect.
$y_{A+g\times sp}$ = value of the response variable (age, litter size, or length) for each area, species, and gender, accounting for the gender-species interaction.

This model is also biologically realistic because it includes the possibility that males in one species may be smaller than females and vice versa. For fecundity, the following model was chosen:

$$\ln y_{A+sp} = \ln \beta_A + \ln \beta_{sp}. \quad (2)$$

An assessment of Cook's statistic for all models revealed no evidence of any highly influential data points. A subsequent analysis of residuals by species indicated, in one case, a potential departure from the assumption that both genders of all species in all areas have the same variance. Highest residuals were reported for age at maturity in *S. acanthias*. Whether these high residual values are truly reflective of the species or an artifact of low sample size is unknown, so I carried forth with the analysis using the above models.

Area effects

Despite the low and unbalanced numbers of comparisons among coarse area designations, inter-regional variation and bias in sampling each life history trait, and the concomitant lack of power to resolve statistically significant relationships across all areas, a general and consistent trend emerged among the five life history traits. Intraspecifically, populations progressed from larger, longer-lived, later-to-mature populations in the northern-most latitudes to smaller, shorter-lived, and earlier-to-mature populations in the mid and southern latitudes (Fig. 2).

Predicting missing life history information by area

In addition to providing a comparison of the area effects on the response variables, the resultant predicting model offers a way to estimate missing life history values by area for each species and gender:

$$y = e^{(\beta_A + \beta_g + \beta_{sp} + \ln \beta_{sp \times g})}. \quad (3)$$

The factors β_g and $\beta_{sp \times g}$ in the above equation are not present in the fecundity predictions.

For both *S. acanthias* and *P. glauca* (Fig. 3), the predicted values mimicked the area trends of the reported values within two standard errors in all but one case (North Pacific *P. glauca* size at maturity was overestimated for both genders), and provided a means to estimate values for each life history by species and gender for areas not yet reported. The outlying cases may indicate an area (the North Pacific) where sampling is not representative of the true population (in this case, of *P. glauca*) and is in need of further investigation.

Cross validating models produced response variables similar to those of the full models in all but two cases (Fig. 4). In both cases (*S. acanthias* fecundity minus the South Atlantic, and *P. glauca* size at female maturity minus the North Pacific), the observed values were in opposite magnitude to that predicted. This difference could reflect either true relationships or possibly indicate areas that are undersampled (i.e., North Pacific for the size at maturity for female *P. glauca*).

Discussion

Knowledge of large-scale intraspecific spatial patterning in life history traits may be important when considering the population dynamics of a species, but such large-scale patterning has seldom been formally explored. Winemiller and Rose (1992) included median and range latitude correlations in their consideration of several life history variables of North American fishes, but comparisons were only interspecific. Vila-Gispert et al. (2002) demonstrated that fishes from higher latitudes north of the equator matured latest and had the highest fecundity, whereas fishes from South America had the lowest fecundity and earliest maturation, although these comparisons were again made interspecifically. Myers et al. (2001) described the relationship between maximum reproductive rate and carrying capacity among 21 stocks of Atlantic cod (*Gadus morhua*) in the North Atlantic using mixed effects models, but their analysis was done for only one species in a limited region. Helser and Lai (2004) also performed a similar analysis for individual growth rates in North American largemouth bass (*Micropterus salmoides*) populations and found latitudinal changes in growth rate.

Regarding elasmobranchs, Cortés (2000) considered trends in intraspecific reproductive traits for sharks but did not explicitly investigate the spatial patterning of those trends. Frisk et al. (2001) found regional differences across five areas for the spiny dogfish using three life history measures (maximum size, and size and age at maturity), but did not specify regional patterns. The authors also performed a similar analysis with several skate species, finding no difference among areas, but they considered only interspecific patterns. Cortés and Parsons (1996) compared the demography of two Floridian populations of the bonnethead shark (*Sphyrna tiburo*), which included several life history measures in the life table analyses, but the small spatial resolution was inadequate to indicate large-scale spatial life history correlations within this species. Lombardi-Carlson et al. (2003) extended the bonnethead shark investigation to a larger portion of the eastern Gulf of Mexico and found latitudinal variation in maturity and size, but again the scale of this study was relatively small. Additional small scale studies on intraspecific geographic variation in reproductive parameters of sharks have been presented by Horie and Tanaka (2002), Taniuchi et al. (1993), and Yamaguchi et al. (2000).

The results of the present study, specifically aimed at sharks as an example, indicate an emerging pattern for intraspecific life history variation, not unlike previously recognized interspecific patterns. Generally, there is a distinct difference in life history traits among areas—a pattern potentially useful when considering region-specific population dynamics. Across taxonomic designations, populations in the northern latitudes tended to be

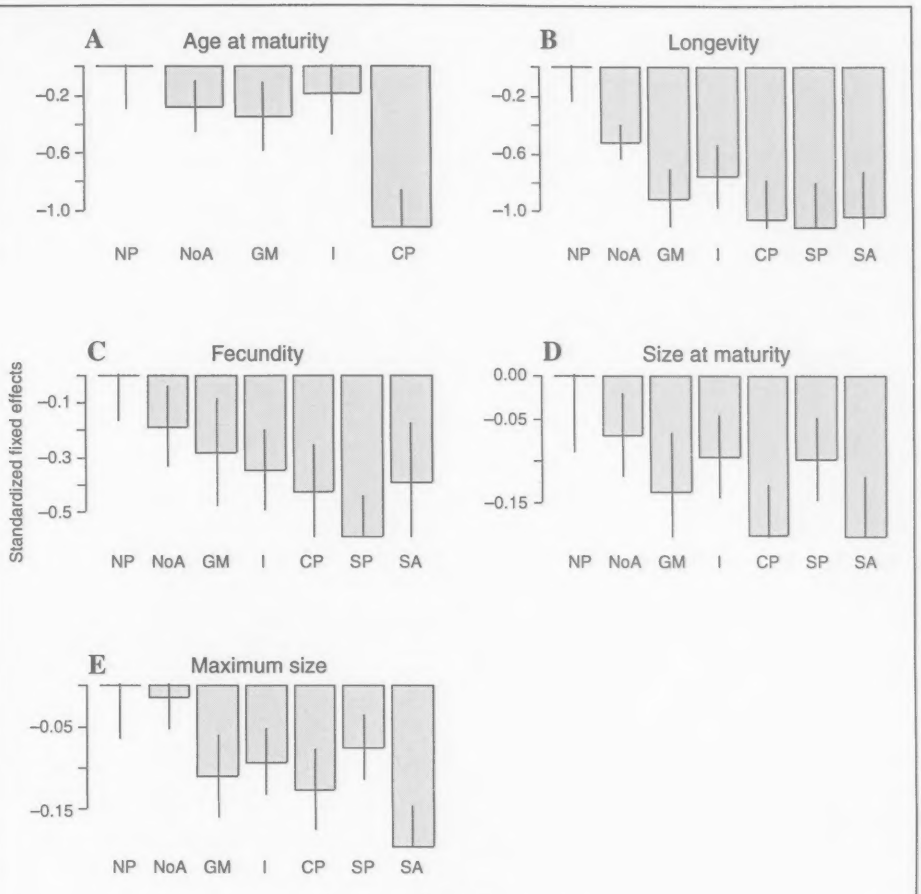

Figure 2

Bar plots of the area effects for each life history trait. Areas: NP=North Pacific; NoA=North Atlantic; GM=Gulf of Mexico; I=Indian Ocean; CP=Central Pacific; SP=South Pacific; SA=South Atlantic. Vertical bars represent one standard deviation. The North Pacific area is the reference area, and all other areas are standardized to it. To interpret the relationships, lower effect values in relation to effect values from another area indicate the life history trait value would also be lower.

larger, to mature later in life, to have longer life spans, and to have greater fecundity compared to conspecifics in the central and southern latitudes. Populations in the North Pacific, in particular, seem to demonstrate dramatic departures in life history measures compared to conspecifics in other areas. Therefore, instead of assuming life history information from one region should be applied to another region, the trends and predictive methods offered in the present study provide a means to extrapolate life history traits of cosmopolitan species in specific areas when only information from other areas is available; this method may prove useful for developing informative priors for Bayesian analyses (Punt and Hilborn, 1997). Caveats to these results include area-specific biases (i.e., certain-size individuals susceptible to capture) and errors in sampling programs and migratory patterns of specific species (e.g., individuals may be found in multiple areas during different parts of their life history). Thus a proper knowledge of the biology of the species is recommended before interpreting the interpolated life history values.

Other factors, such as fishing pressure, may influence regional differences in life history traits, challenging the interpretation of such patterns. Truncation of size and age classes, and reduction in age at maturity are recognized byproducts of heavy fishing (Longhurst, 1998a; Rochet, 2000). Although all the populations used in this study are and have been fished—some more intensely than others—this study assumes there is no consistent pattern to such exploitation in shark

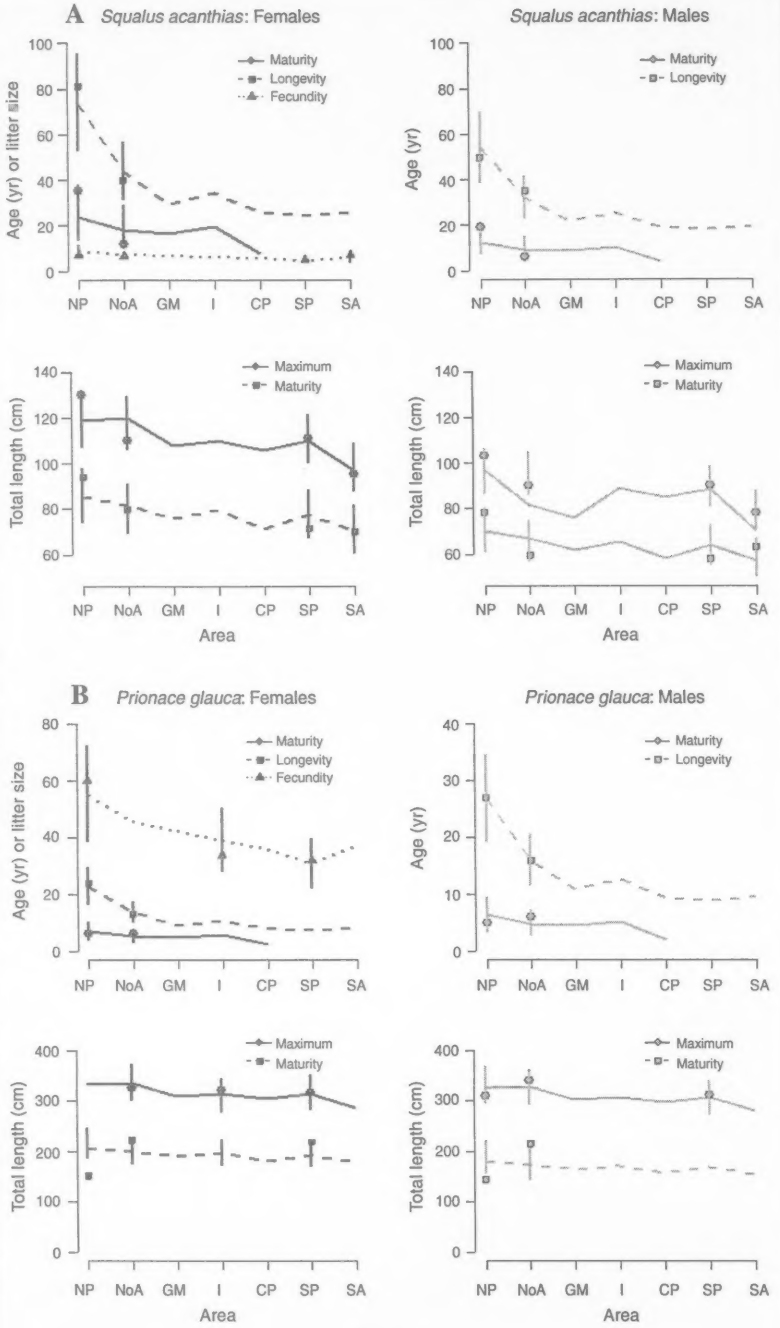

Figure 3

Example of predictive plots based on the results of the generalized linear models for each life history trait by gender for two species: (**A**) *Squalus acanthias* and (**B**) *Prionace glauca*. Lines represent predicted values and symbols represent values from the literature. Vertical lines represent two standard errors.

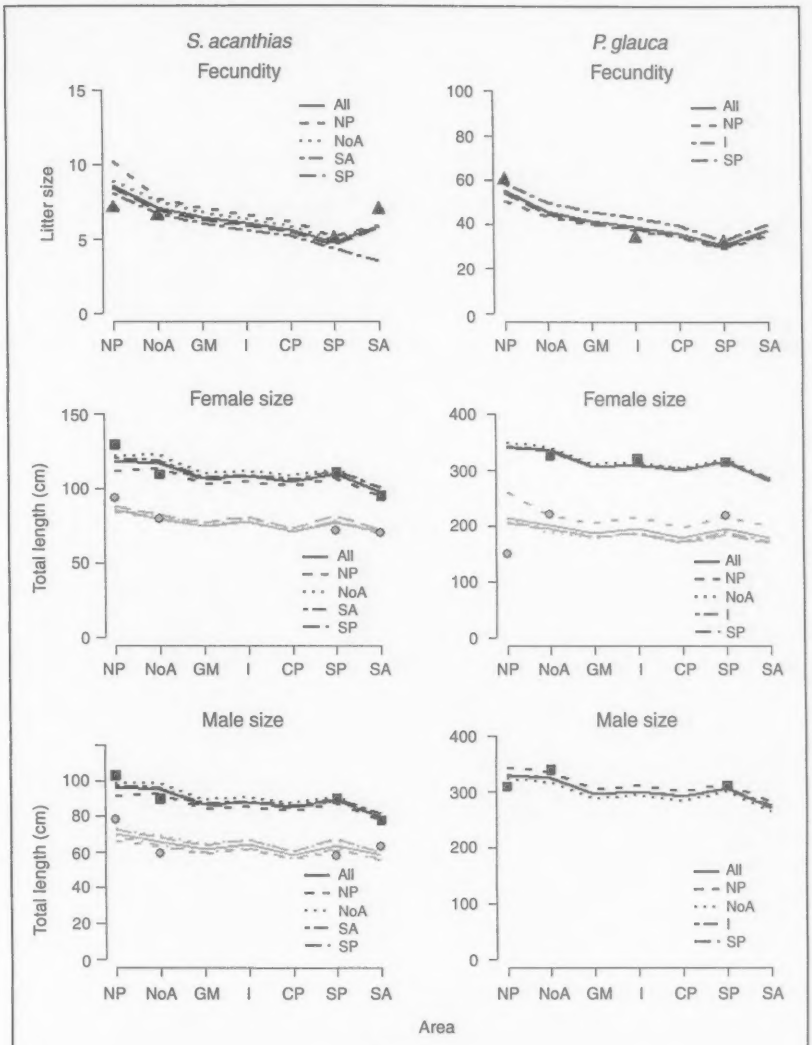

Figure 4

Plots of model predictions derived by cross validation for *S. acanthias* and *P. glauca*. Lines representing predicted value are distinguished as follows: "All"- all areas are included in the prediction; Lines denoted by area are predictions made without that area. Literature values are indicated as follows: triangles— fecundity, circles— size at maturity, squares—maximum size. Size at maturity for male *P. glauca* was not included because of insufficient data. Areas: NP=North Pacific; NoA=North Atlantic; GM=Gulf of Mexico; I=Indian Ocean; CP=Central Pacific; SP=South Pacific; SA=South Atlantic.

populations that would explain the results. Specifically, there is no reason to believe that species in the southern hemisphere have been more heavily fished than conspecifics in the northern hemisphere.

The results offered in the present study are based on small sample sizes in most areas, but hopefully they will bring attention to the usefulness of collecting spatially varying intraspecific information with the idea of constructing more robust models. Most investigations describing the patterns of shark life history traits suffer from insufficient biological resolution (either temporally or spatially) of the very parameters and subsequent relationships they attempt to explain (Smith et al., 1998; Cortés, 2000). Limited biological knowledge and

subsequent high uncertainty in the estimation of vital rates of many marine species, including elasmobranchs, is testimony to the fact that the accumulation of life history information should be a priority to biologists, fisheries scientists, and resource managers. Results from the models presented here could be used to hypothesize life history values in areas currently lacking information and thus be tested with further sampling in those areas. It is also hoped that the approach offered here may indicate areas where sampling may not be sufficient, as denoted by departures from the general model trend. Targeted sampling in that area would help resolve whether the departure is from a true area effect or species effect. As more data is gathered, it will be possible to explore other factors—such as temperature and guilds (e.g., coastal versus oceanic)—in the model structure.

Once steps are made to further resolve the species and area effects, one may start to ask questions regarding the cause of particular area effects. Potential mechanisms of true coarse-scale gradation of life history traits may be contained within the generalized characteristics of oceanic zoogeographic realms (Longhurst, 1998b), although a slightly less abstract mechanism could be found in the physical forcing events that characterize regions in the northern and southern hemisphere. Although both hemispheres demonstrate similar large-scale current and wind patterns, physical forcing events tend to be stronger in the northern hemisphere (Trenberth et al., 1998). Because this study offers coarse area designations to intraspecific life history variation, it is most likely a product of some macroscale characteristics of each region. Attention should therefore be directed towards large-scale characteristics of each region to explain these patterns, although small-scale dynamics are important for understanding each population's specific response to local environmental conditions (i.e., countergradient variation in growth rates [Conover, 1990; Conover and Present, 1990]).

It is becoming increasingly important to be able to assess fish stocks with minimal data. By combining genetic data revealing differing levels of intraspecific population substructure with the increasing number of studies demonstrating localized adaptations and plasticity in population parameters, it is apparent that intraspecific spatial differences must be considered in species management (Avise, 2000; Roff, 2002). Although the predictive power of this study may currently be weak because of low sample sizes, it offers a method to quantify potential spatial patterning in intraspecific life history traits that may allow responsible management of regionally data-poor species, and it may help frame future sampling protocols and studies of spatial patterns in life history traits.

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Appendix

Life history traits and area assignments for species used in the analyses. Sizes are total length (cm). Areas: NP=North Pacific; NoA=North Atlantic; GM=Gulf of Mexico; I=Indian Ocean; CP=Central Pacific; SP=South Pacific; SA=South Atlantic.

		Maximum size		Size at maturity		Age at maturity			Longevity	
Species	Area	F	M	F	M	F	M	Mean fecundity	F	M
Carcharinus acronotus	GM	130	122	110	103	3	2.5	5		
Carcharinus acronotus	NoA	154	164	120	110	4	3	5		
Carcharinus amblyrhynchos	CP	190	185	137	132.5			5		
Carcharinus amblyrhynchos	I	178	168	135	130			3		
Carcharinus falciformis	GM	308	314	235	217.5			11		
Carcharinus falciformis	NoA	330		234	218			10		
Carcharinus falciformis	I	283	244	248				11		
Carcharinus falciformis	SP	250	225	200	212.5			7		
Carcharinus leucas	GM	285		225	215			8		
Carcharinus leucas	I	300		239.5	239.5			9		
Carcharinus limbatus	GM	191	175	156	133	7	4.5	4.9	10	9
Carcharinus limbatus	NoA	202	189	156	143.5			4		
Carcharinus limbatus	I	247	246	208.5	201.5	7	6	6	11	10
Carcharinus longimanus	NoA	260		175				12.5		
Carcharinus longimanus	SA	250	235	185	185				17	14
Carcharinus longimanus	I	270	245	185	198					
Carcharinus longimanus	SP	270	251	200				6		
Carcharinus longimanus	CP	272	240	182	182			7	11	11
Carcharinus obscurus	NoA	371	360	284	279	21	19	11	39	39
Carcharinus obscurus	I	389	324	300	280	24	20.5	9.9	34	
Carcharinus plumbeus	CP	190	172	144	131			5.5		
Carcharinus plumbeus	NoA	234	226	183	180	15	15	9	32	40
Carcharinus plumbeus	I	199	190.5	169	167			8		
Galeocerdo cuvier	NoA	450		317.5	310	10	10	55	16	15
Galeocerdo cuvier	GM	450				8	7		11	9
Galeocerdo cuvier	I	410	370	340	290			35		
Galeocerdo cuvier	SP	428	350	287				31		
Prionace glauca	NP		310	150	145	6	5	60	24	27
Prionace glauca	NoA	327	340	221	215	6	6		13	16
Prionace glauca	SP	316	312	218				32		
Prionace glauca	I	321.5		214				34		
Rhizoprionodon taylori	SP	78	69	54	56			4.5		
Rhizoprionodon taylori	I	66	55	45	43			5		
Sphyrna lewini	GM	310	300	250	180	15	10		17	12
Sphyrna lewini	NP	324	305	210	198	4.1	3.8	26	14	11
Sphyrna lewini	I	346	301	200	150			16.5		
Galeorhinus galeus	NP	195	185	180	175					
Galeorhinus galeus	SP	174	175	140	135	15	17	28.4	53	41
Galeorhinus galeus	SA	155	148	128	117	17.5	13	23	36	36
Isurus oxyrinchus	NP	351		280		16			34	
Isurus oxyrinchus	SP	340	270	280	195			9		
Isurus oxyrinchus	NoA	375	298	280		14		13.5	23	9
Isurus oxyrinchus	I	333	271	266	199.5			11.5		
Alopias superciliosus	NoA	444	410	341	276			2		
Alopias superciliosus	NP	422	357	341	288	13	10	2	20	19
Squalus acanthias	NP	130	103	94	78.5	35.5	19	7.1	81	50
Squalus acanthias	NA	110	90	80	59.5	12.1	6	6.6	40	35
Squalus acanthias	SA	95.5	78	70	63			7		
Squalus acanthias	SP	111	90	71.5	58			5		
Squalus mitsukurii	NP	114	94	97.5	70	22	12	8.8		
Squalus mitsukurii	I	95	81	69	60			6.4		
Squalus mitsukurii	CP	91	82	69	48	15	4	3.6	27	18
Squalus mitsukurii	SP	104	102	85				3.5	16	14

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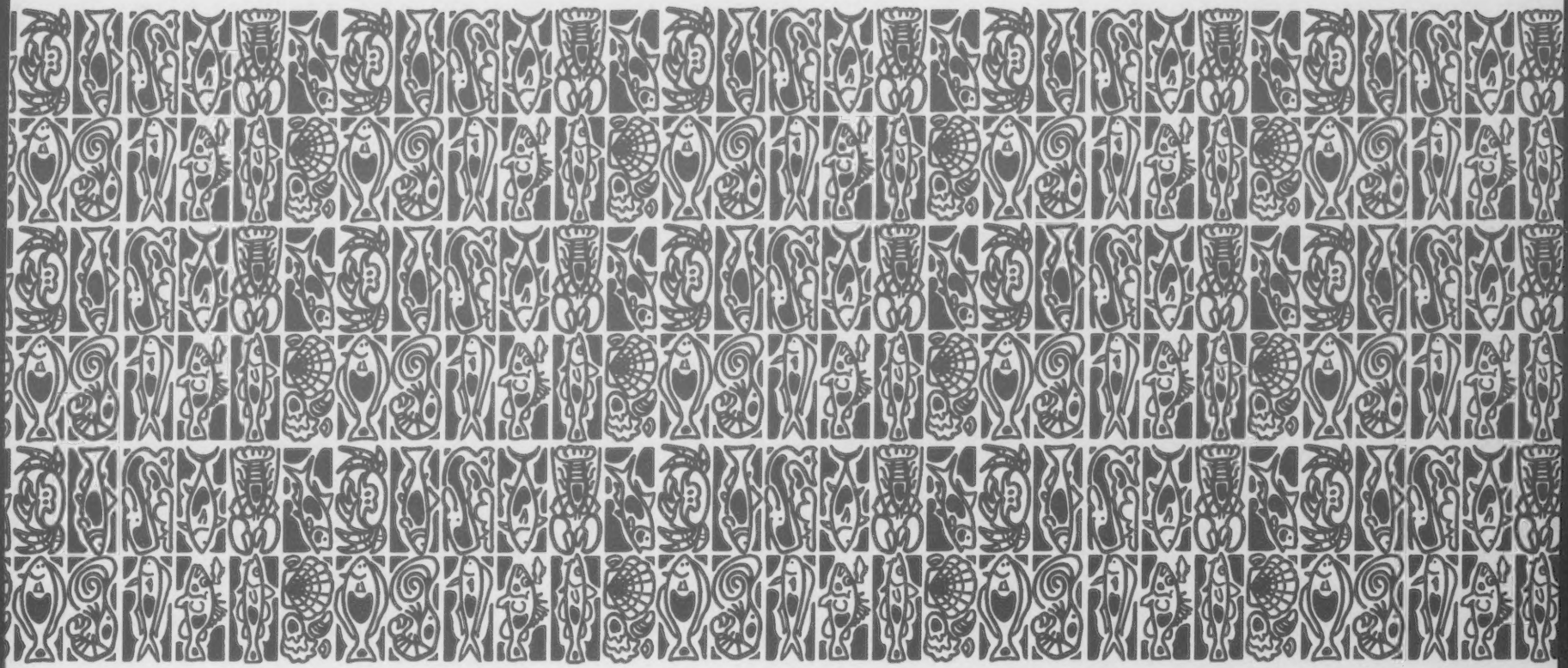
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